PRACTICAL PHOTO-MICROGRAPHY

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PRACTICAL PHOTO-MICROGRAPHY

BY

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First Published 1911
Second Edition 1925
Third Edition 1936

Printed in Great Britain by
Butler & Tanner Ltd., Frome and London

PREFACE

In presenting the third edition of this book, a decade after the second edition, the authors have endeavoured to eliminate any matter that might now appear unnecessary and have brought the description of apparatus up to date. The subject dealt with is essentially a practical one, and the intention has been to describe the procedure from that standpoint. Recently there has been a tendency to elaboration of apparatus and the design of some microscopes has been altered considerably. The main purpose of elaboration has been to enable less skilled or less specialized workers to obtain results quickly and easily. This applies particularly where such apparatus is installed in research departments of commercial undertakings or in large institutions. It must, however, be recognized that elaboration of apparatus cannot replace skill, nor can it enable any user to obtain better results than can be achieved with simple appliances. Its very complication may be a hindrance under unfavourable conditions of work. The writers, therefore, have been much more concerned with description of methods and the principles involved than in repeating the catalogue descriptions of ingenious appliances. Some parts of the text have been rewritten and considerable additions made which it is hoped will increase the value of the book. More attention has been given to the photography of metal surfaces and similar opaque objects. In this connection we are greatly indebted to Mr. H. Wrighton of the Research Department, Woolwich Arsenal, both for the excellent photo-micrographs included in the plates and for his help and advice in the preparation of this part of the text. A short section on the use of infra-red radiations and a new chapter on photo-micrography in the ultra-violet region have been added. The latter is not intended to be a complete account either of the method or of the apparatus, but it is enough to enable those interested to understand the principles involved, and the purpose of the use of radiations of short wave-length.

We are indebted to many firms, both English and Continental, for the use of illustrations and for the loan of blocks, and to all these we wish to express our thanks. We are also greatly indebted to Dr. W. J. Purdy for the help he has extended to us in reading the proofs, and for many valuable suggestions. There are few references to the works of others, but this does not mean that we are unaware of the efforts of other workers or that we wish to ignore them. The book is mainly an account of the methods and processes we ourselves use, and in some cases have evolved. From this standpoint it should be regarded, and it can only be hoped that it may be of service to those interested in photo-micrography.

J. E. B.
F. V. W.

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PRACTICAL PHOTO-MICROGRAPHY

CHAPTER I

INTRODUCTORY

As an instrument of research, either in its application to scientific work, or in its varied uses where scientific methods have been applied industrially, the microscope holds a pre-eminent position. It was early recognised that some means of recording the varied appearances of microscopic images was desirable, and for many years no method but that of hand drawing was known. With the advent of photography these conditions were changed, and it was recognised that the combination of photography with the microscope had great possibilities, and opened up a fresh field for work.

Photo-micrography has become, therefore, an important branch of microscopic work, but it still suffers from certain disabilities. When a microscopist is observing an image under the microscope, he can so focus any particular plane of the object as to bring out the part he wants. In a drawing he can delineate this part only, suppress portions of the image or entirely eliminate any ill-defined areas. In viewing an object each successive plane can be focussed, and so a general idea of its structure in depth is obtained.

The quality of penetration, or the power of showing more than one optical plane at a time, is not evident in any first rate high-power objective: in fact, the higher the quality of the objective, the less depth of focus it possesses. The photographer has difficulty in embodying these appearances in his picture, and this now constitutes the chief, if not the only, objection to photography as applied to the microscope.

Photo-micrography may be approached from either of two sides: by the microscopist who desires a method of recording his observations, or by the photographer who has exhausted his interest in landscape work, portraiture, or what is usually referred to as ordinary photography, and is looking for a new field of work.

The microscopist will certainly have by far the easier task, as the difficulties encountered are chiefly those of the proper use and adjustment of the microscope rather than mastering of purely photographic processes. The latter are now greatly minimised as the result of the constant simplification of photography.

On the other hand, the photographer who wishes to enter upon a new field will find that in order to attain any degree of success he has to master the use of the microscope as an optical instrument, and to acquire a general knowledge of the objects which he wishes to photograph.

The earliest examples of the application of photography to the microscope are coincident with the days of the daguerreotype: so that it started from the earliest times when photography was practicable at all. Then, however, the microscope was in a comparatively rudimentary condition—particularly so far as its optical parts were concerned. In recent years so much progress has been made in the preparation of specimens for the microscope, and in methods of staining, that photo-micrography has steadily improved in status. Also the reproduction of photographs, whether microscopic or otherwise, for the purpose of book illustration, is now so good that this method of delineation has become possible for subjects which at one time could not have been dealt with at all. Recently, too, direct photographic prints have been used for illustrating scientific papers where very fine detail has to be shown. One example of this method is included in the collection of typical pictures contained in this book.

Photography itself, however, suffered from considerable disabilities, and for a long period was surrounded by technical difficulties. The skill and experience needed to work successfully the only process then available, the so-called ‘wet-plate’ process, hindered any but expert photographers from attempting it. Consequently photo-micrographic work was only practised by the few, although it is unquestionable that the photographs then produced by two or three well-known workers have seldom been equalled, and in their class have never been excelled. With the advent of the ‘ dry-plate ’ method all this was changed, and photo-micrography began to take up the position in microscopy that its earliest devotees expected.

The difficulty already mentioned, of delineating photographically an object, the details of which extend through a series of planes, is certainly a considerable one, and in the majority of cases almost impossible of solution. It is, however, quite possible to select planes that give the best general appearance, and, by methods which will be described hereafter, to select the focus which is most suitable, and to exclude that part above or below which is useless. The objects to which this objection applies are comparatively few, particularly with the present methods of section-cutting and staining. Where the sections themselves are thin, the difficulty will not appear at all.

Photo-micrography now embraces a wide field of work. It is applied particularly in pathology, bacteriology, and histology ; but in later years its application to metallurgy and petrology has opened up a still larger field. It is now used in all cases where a microscope is employed.

Its advantage over any method of drawing must be obvious, as it is impossible to get an appearance on the photographic plate, assuming of course that the work is carried out honestly and with due regard to the principles involved, other than that in the object itself. By its use, too, the factor known as ‘ the personal equation ’ is eliminated, so that in recording the results of research only a truthful rendering of objects is possible. Many objects, either from their intricate structure or fineness of detail, cannot be drawn successfully : but by the photographic method no detail which can be seen is too fine to be delineated with perfect accuracy.

To acquire any considerable degree of dexterity a sound knowledge of the microscope, from its optical as well as from its practical side, is essential, and in addition the technicalities of pure photography should be thoroughly mastered.

The microscopist, who is anxious to start this work, cannot be too strongly advised first to acquaint himself thoroughly with the processes of ordinary photography. For this purpose a considerable amount of work should be done with a camera in the field and, if possible, in photographing such objects as flowers and still-life subjects with a view to correct colour-rendering in monochrome. No mere experience with a camera of the snapshot variety is of the least assistance: in fact, it is almost worse than useless as no idea whatever is obtained of the value of the light being used, nor are the rendering of colour and the differences in tone which occur in photography ever appreciated.

The photographer on the other hand should master, with a simple microscope, the ordinary methods of microscopic work; he will then more easily apply his photographic knowledge in recording what he sees.

Until comparatively recently there was a large amateur element who practised the photo-micrographic art purely for its own sake. An active society, the 'Photomicrographic Society' still exists which owes its vitality largely to such workers, but this element tends to diminish in numbers. On the other hand, much more is now done in laboratories and research institutions generally. The amateur regards it as worth while to spend much time and labour in making a preparation that will yield a good photograph, regarding the latter as an end in itself. The laboratory worker has an entirely different outlook; often he has to photograph an object made with no regard to permanency or technical perfection but which does demonstrate some diagnostic characteristic or stage in a research. Thus the purpose of a photo-micrograph is to secure a permanent record of any appearance that is of more than passing interest. For such purposes simple appliances have been devised and are described later. It is however rarely possible under such circumstances to secure the best results, and for this reason more than any other the standard of such work does not tend to rise. To obtain a fine photo-micrograph a first-rate specimen is essential and this is not always available.

CHAPTER II

THE MICROSCOPE

Microscopes have not altered in general design for very many years, and up to comparatively recent times there were two well-defined types: the one generally known as the English and the other as the Continental model. In the English models the body part is supported on a tripod foot which has a considerable spread. The result is that English microscopes are usually rigid and stable whether in a vertical, inclined, or horizontal position. In the Continental models the body is supported on one metal casting, which consists of a horse-shoe foot with two vertical projections; between these projections the body can be swung, a pin or rod passing through and allowing inclination about this point as an axis.

In choosing a microscope for photography the following points must be considered, and indeed are essential for any serious work. The foot should be heavy and should rest on three points which should be widely separated; in fact, the instrument should be so built that it requires no clamping down to its support when the body is in a horizontal position. The body-tube, containing the eye-piece and objective, should have a coarse adjustment actuated by rackwork, moving the tube to and fro with precision. There should be no evidence of backlash —that is, inclination for the teeth on the wheel that engage in the rackwork to shake or fail to move the tube the moment the head of the focussing screw is turned. The action should be such that the body-tube moves easily, yet comes to rest directly the movement of the milled heads is stopped. A good coarse adjustment has a motion responsive enough to bring an object into approximate focus with an oil-immersion objective, so that only a final touch to the fine adjustment is necessary for perfect focussing.

The rackwork should move the body-tube in the direction of the optic axis only; if an object on the stage is observed while the rack is moved, there should be no evidence of lateral displacement of the image.

The fine adjustment is of primary importance, and any want of precision in its design or construction renders an otherwise good instrument practically useless. There are many types of fine adjustment: in fact, each maker pins his faith to some particular method, but it is not within the scope of this work to go into the details of design. The mechanism should be tested by putting an object on to the stage of the microscope, and an observation should be made with an objective of high power. The fine adjustment screw should be lightly but firmly grasped on either side by the thumb and, preferably, the second finger, so that an even pressure is exerted without strain. On turning the fine adjustment screw no evidence of movement of the object should be discernible: it should simply come into and go out of focus as the screw is gently revolved. It is necessary for this movement to be slow consistent with convenience in working, since owing to the large aperture of the objectives now obtainable small focal changes have a greater effect on the image. It is necessary that a fine adjustment shall show no trace of movement after the precise position of correct focus has been secured, and that it shall remain so for any required length of time.

The microscope should be provided with a draw-tube, preferably actuated by rackwork, although this is not absolutely necessary; the length of the draw-tube will be dependent on the objectives that are selected, but it is preferable to have sufficient length of tube so that either 'short' or 'long' tube objectives, as they are called, can be used at will. This entails an alteration of length from 150 to 270 millimetres, an extension that is best obtained by using an additional tube which itself is removable from the microscope. Such a tube is conveniently 100 millimetres long so that the ordinary sliding tube on the microscope gives the necessary range for adjustment. If only objectives corrected for 160 or 170 mm. tube-length are to be used, a range of 20 mm. on the draw-tube on either side of the correct setting is sufficient. This is to provide a correction for varying cover-glass thickness, a point that will be referred to later. A body-tube of large size is desirable, instruments designed for photo-micrography commonly have an outer tube of 2 inches diameter. This is to provide against internal reflections, particularly when using long-focus lenses without oculars. If the microscope has a body-tube of small diameter it is advisable to insert a lining of dull black paper or preferably of black velvet with the pile inside.

The stage should be large, and very firmly supported, so that no light pressure on it—such as occurs when an object is being moved about on the stage—will result in flexure and consequent displacement of the object in relation to the optical system. The stage should have a large hole in it; or, better still, an elongated slot with its opening on the side away from the observer. The opening should in any case be sufficiently large to allow the use of any sub-stage illuminating apparatus of ordinary pattern.

A mechanical stage is very convenient, but by no means essential. If, however, it is adopted, it should be of the best possible design and construction: a bad mechanical stage is worse than useless. It should work quite smoothly, and exactly at right angles to the optical axis. If the moderate additional expense is not a consideration, verniers for determining the exact position of any particular spot in an object, so that a record of its position may be kept, are often of great utility. Except for high-power work, or where long searches for particular appearances are necessary, the writers prefer to use an instrument with a large flat stage, and without any mechanical arrangement for shifting the object; certainly, unless a mechanical stage of the best make can be obtained, this is the better method. A centring stage—that is, one in which an arrangement is made for a very small movement of the stage to facilitate the exact centring of an object—is exceedingly useful in setting up objects for photographing. A fault that is often found in microscope stages of all kinds is that they are not mounted on the microscope limb truly at right angles to the optic axis. If the error is large it is evident at once if a flat object is observed with an objective of medium power: it will be impossible to bring opposite edges of the field into focus at the same time. If the error is smaller it will still show itself in photographs taken with high powers, thus a negative will always fall off in definition in one direction. This defect can occasion considerable annoyance and uncertainty ; every other part of the equipment can be in perfect adjustment, yet the results will be unsatisfactory.

The points so far enumerated are the principal ones in the part of the instrument that lies above the stage ; but the sub-stage apparatus and the means for adjusting it are equally important. Any instrument for photo-micrography must have a sub-stage condenser, and, as will be found later, the proper use of this is only second in importance to the management of the objectives themselves. The arrangement for carrying a condenser should be thoroughly stiff and solid ; the remarks made about the perfect alignment of the stage equally apply to the mounting of any sub-stage fittings. The condenser is carried in a fitting which has freedom of movement in the direction of the optical axis and which is actuated by rackwork for exact focussing and adjustment. It, too, should be thoroughly tested to make sure that the movement is a regular one, without lateral displacement, and that the rackwork has no backlash or stiffness in action. In some stands a fine adjust-

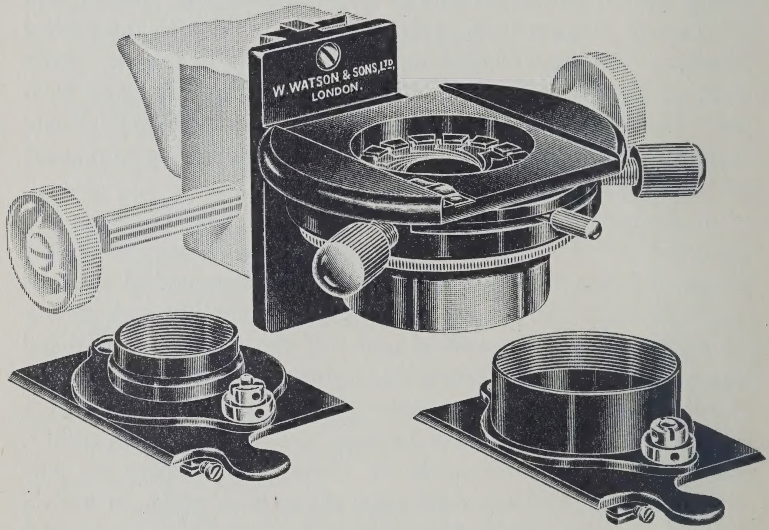

Fig. 1.

ment is also provided, exactly as used for focussing the objective; but the writers have never felt the need for this, and regard it as an unnecessary complication. A good rackwork motion, used with care, will fulfil every requirement. The condenser should be that known as the 'centring' type—that is, it should have an arrangement for altering its position in a direction at right angles to the optical axis, so as to get it in perfect alignment with the axis of the instrument. This is usually effected by having two screws which engage the mount carrying the optical portion of the condenser (Fig. 1). On the side opposite to that on which the screws are situated is a spring which keeps the mount always up to its position. The inner ring carries the optical portion and is movable by turning either or both screws in relation to the fixed collars above and below. Underneath the condenser an iris-diaphragm is mounted so that the available aperture of the condenser system may be altered at will. In some Continental stands there is an arrangement for throwing the iris-diaphragm out of the optical axis of the microscope so that oblique illumination may be obtained, but such an arrangement is of little use for ordinary work, useful though it may be for objective testing.

A convenient method of interchanging sub-stage fittings is by means of that known as an 'Akehurst' changer. Each optical part is fixed to a dovetail carrier on which it is centred by means provided (Fig. 1). This slide fits loosely into a female dovetail slide and is clamped when in position. It is therefore possible to have two or more sub-stage illuminators interchangeable at will, but the centring screws already described are still necessary to finally adjust the condenser for each change of objective.

For metallurgical and petrological work a rotating mechanical stage is almost essential, as it is necessary to be able to vary the direction and the incidence of the light in relation to the objects.

The following illustrations of microscopes are chosen to indicate types that are at present being made by some of the leading manufacturers in England and abroad. They are not selected as being necessarily the only ones suitable for the purpose in view. It may be taken as certain in purchasing a microscope that the best will be found the most satisfactory, although it need not be implied that an expensive stand is a necessity. There are few really bad microscopes made nowadays; even the low-priced ones, as the result of competition, have to be well finished, and, if bought from a maker of repute, will usually give satisfaction.

The instrument selected will largely depend on the work that has to be done. If the use of high powers is contemplated, an instrument of the highest class is essential; but for ordinary work there are many microscopes available of moderate price. The writers used for some years a microscope of the Wales type, made by Messrs. Swift & Son, and found it in every respect satisfactory. It was first adopted because of its being so highly recommended by that pioneer of photo-micrographic work, Mr. Andrew Pringle, who referred to it particularly in the earliest editions of his book on photo-micrography. As the writers have found this microscope equal to any requirement, they feel justified in giving some special description of it, for it will meet the needs of the majority of photo-micrographers.

The chief advantage of this form of stand is that the centre of gravity of the instrument is practically constant at whatever inclination it may be used. As will be seen by the illustration the inclination of the body is effected not by swinging on a central pivot, but by the movement of a semicircular portion of the body part between two jaws, and these can be clamped by a clamping-screw so that the microscope is rigid in any position. The base of the stand has a wide spread, so that the instrument is stable and well balanced; the fine and coarse adjustments are entirely satisfactory, and have worked with precision over a period of some thirty years. The stage, as shown, is of a mechanical type, and can be completely revolved; the screws for actuating it are conveniently placed whatever the position of the stage. The sub-stage condenser has centring-screws, with rack and pinion adjustment for focussing. The mirror is carried on a swinging arm of sufficient length to enable it to be brought well above the stage if necessary; and this will be found of use when opaque objects are to be illuminated. The instrument has been in constant use for widely varying classes of work, and has fulfilled every requirement.

Recently the same firm has introduced an improved model embodying the same principles. It is known as their Premier microscope (Fig. 2) and is one of the most complete instruments now on the market. The whole construction has been redesigned with a view to securing greater stability, the radial arm being thicker and the stage and sub-stage having every necessary adjustment. It is an expensive model, but in cases where cost is a secondary consideration it does represent one of the best to be obtained.

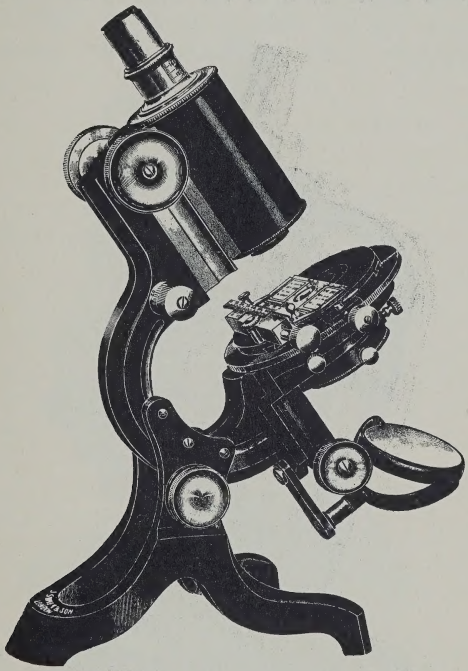

FIG. 2.—Premier Microscope. (James Swift & Son, Ltd.)

The New B.L.M. Microscope (Fig. 3), by Charles Baker of London, is a typical English model with large body-tube, inside which a diaphragm is fitted to stop internal reflections. The limb is of a girder pattern to secure rigidity and is in one piece. It has a rotating mechanical stage, centring sub-stage and swing-out condenser. The cost is moderate.

The Massive Model Microscope (Fig. 4), by R. & J. Beck, Ltd., of London, was designed for and with the assistance of the National Institute for Medical Research; it has been made throughout on a very heavy and stiff design, so that no vibration or flexure can take place. The limb consists of a massive brass casting which extends in one piece from the body to the mirror. The tail-piece and fine adjustment slide are planed out in one continuous cut so as to ensure the perfect alignment of the sub-stage with the focussing adjustment. The stage, which is strengthened below by side ribs, is screwed and soldered on to the limb so that it is as strong as a solid piece. The pillar and base are equally heavy and free from any spring. The fine adjustment is exceptionally delicate, each division on the milled head being equal to 0·001 mm. The coarse adjustment milled heads are large, giving an accurate movement with all but the highest powers. The square stage has two dovetailed grooves planed in its surface and a mechanical stage moves in these grooves or can be removed at will. This mechanical stage has its actuating milled heads pro-

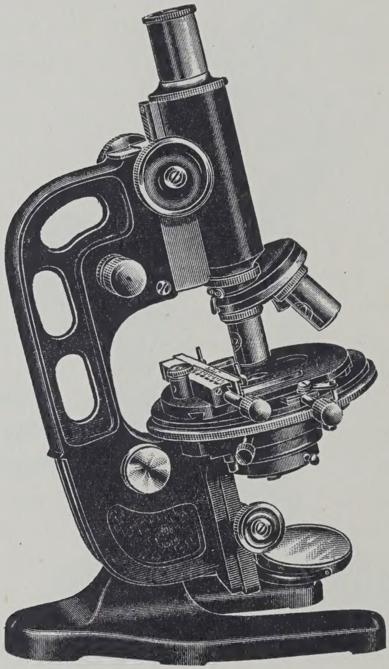

FIG. 3.—New B.L.M. Microscope. (C. Baker.)

jecting horizontally on the right-hand side. The upper one moves the slide laterally and has 3 inches (75 mm.) travel, the lower one moves the slide vertically and has a travel of $1\frac{1}{4}$ inches (30 mm.). This motion is provided with a clamp screw so that it can be locked to prevent any chance of the slide moving when the instrument is in a horizontal position. Verniers reading to 1/10 mm. are provided in convenient positions for reading.

The sub-stage racks up and down on the lower portion of the limb accurately in the optic axis, and the mirror fits by means of a sliding fitting on the same slide. The sub-stage has centring adjustments and is of the R.M.S. standard size, but at its upper end is fitted with a dovetailed fitting to receive the condensers or dark ground illuminators. All the illuminators are mounted on Akehurst changers which slide easily into the fitting and are held accurately in position by a clamping screw. The illuminators are accurately centred and of the same length, so that they can be interchanged without removing the object. The back of the foot of the microscope carries a short vertical post, and when the microscope is in a horizontal position for photo-micrography, this takes the weight of the limb and forms

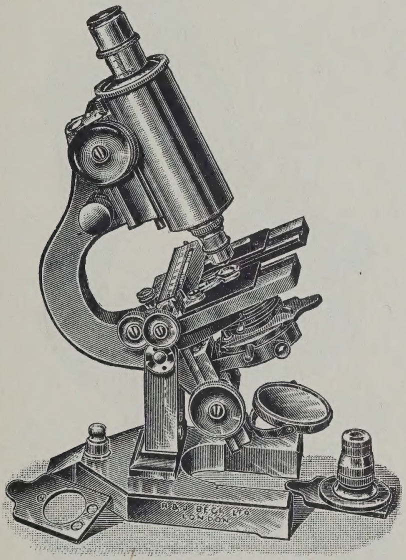

Fig. 4.—Massive Model Microscope. (R. & J. Beck, Ltd.)

a rigid support. The body-tube is 2 inches in diameter, with a draw-tube giving a variation in length from 140 mm. to 200 mm. A rack and pinion double extension draw-tube can be fitted if desired. A circular rotating stage can be supplied in place of the square stage, but in this case some of the advantages of the interchangeable sub-stage apparatus are lost. With this microscope the body and apparatus can be relied upon to be truly in the optic axis, the manipulation of one part of the instrument does not tend to upset the adjustment of the other parts.

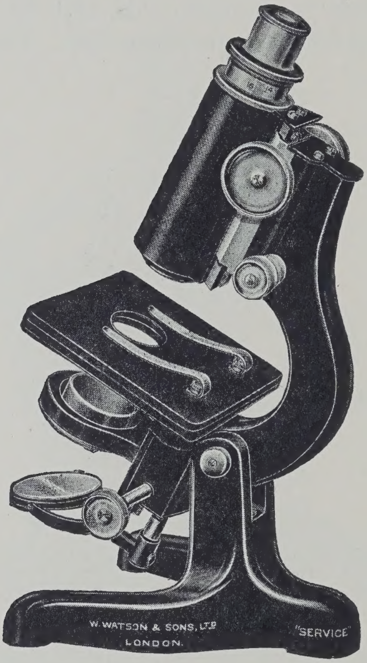

FIG. 5.—The 'Service' Microscope. (W. Watson & Sons, Ltd.)

The 'Service' Microscope, made by W. Watson & Sons, 313, High Holborn, W.C., is an example of a simple yet efficient type (Fig. 5). It conforms to the specification of the British Science Guild, and is designed so that additions may be made to it without sending it to the makers. It is sufficiently stable for photo-micrography, and is steady at any angle of inclination. The sub-stage apparatus is carried on an extension of the limb, not on the under side of the stage as in many of the less expensive stands. The stage is square and spacious; a mechanical stage of special design is easily added, and a centring sub-stage is supplied if required. This microscope is a good example of specialised machine-tool production.

The modern method of machining the limb which carries the optical parts ensures perfect alignment and is shown graphically in Fig. 6. The limb, as shown in the illustration, carries the body at one end, the sub-stage at the other; between are the supporting bracket pieces for the stage, and at its extremity is the mirror fitting.

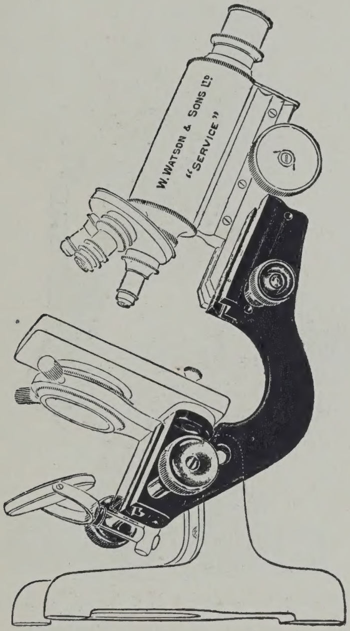

FIG. 6.

The dovetailed fittings which carry the body and those which carry the sub-stage are machined in one continuous operation ensuring basic alignment throughout. Simultaneously the stage brackets are machined to ensure that they are truly at right angles to the body and sub-stage. A high degree of accuracy is thus assured.

The microscope body has hitherto consisted of the outer tube together with a fitting which screwed into the lower end of the body-tube and was threaded to receive the objective. This body-tube was fixed to the bar on which the dovetails were machined, to form the coarse adjustment, the fixing of the body-tube and bar being done either by screws alone or by screws and solder.

The body-tube, the objective fitting and the bar are now machined out of one solid piece of metal and the risk of the parting of either portion is abolished. Parallelism between the optical and mechanical axes is ensured. The body-tube is 40 mm. diameter.

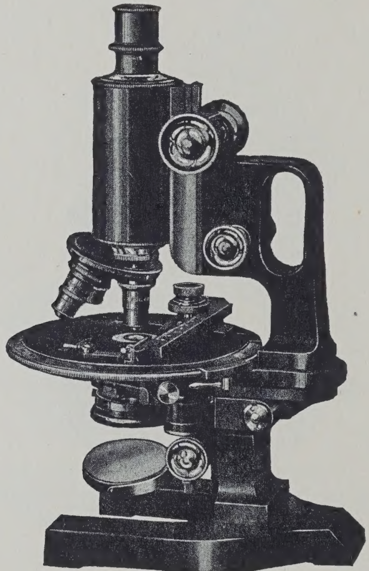

Fig. 7. The Spencer Microscope No. 5. (Hawksley & Sons.)

Messrs. Watson & Sons also supply their well-known Van Heurck and Royal models, which embody every refinement.

Among American microscopes those made by the Spencer Lens Company and sold by Messrs. Hawksley & Sons, Wigmore Street, London, are of good design and construction. The Spencer Microscope, No. 5 (Fig. 7), is particularly stable owing both to its solidity and to its low centre of gravity. The tube is large and the fine adjustment gives a movement of one micron for each division on the milled head. The stage is of large size, and is mechanically operated by concentric milled heads on one axis. This method does not at once appeal to those unaccustomed to such an arrangement, but it will be found convenient in use as the fingers have only to be moved slightly up or down to operate either movement of the stage. The substage is somewhat cramped, but it will take most ordinary illuminating appliances.

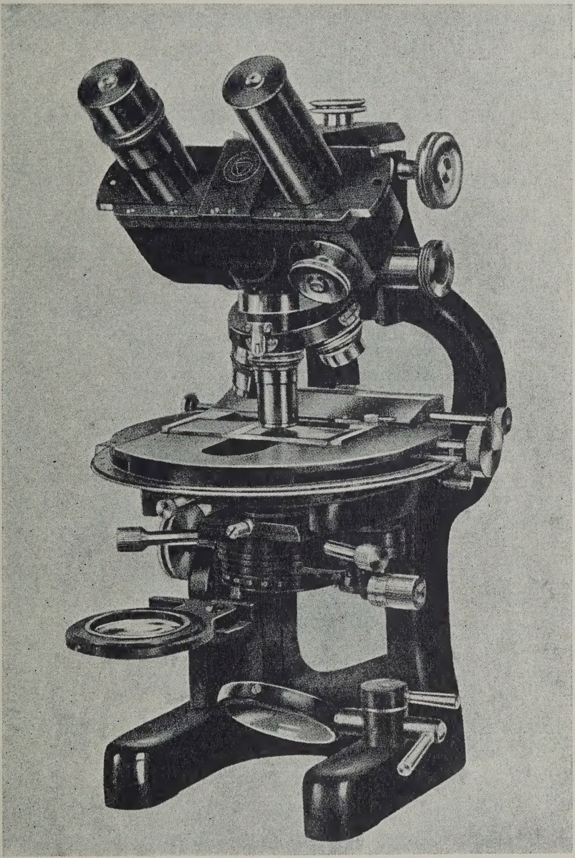

FIG. 8.—The 'New Research' Microscope. (The Bausch and Lomb Optical Co.)

The Bausch and Lomb Optical Company of U.S.A. have introduced a 'New Research' microscope which is particularly suited for photo-micrography and is of novel design (Fig. 8).

It is primarily a binocular but can be converted to a monocular instrument by exchanging the body-tubes. It is not inclinable, but to use it in a horizontal position the entire instrument is placed on its back resting upon three bosses provided on the two arms and base. It is obvious that great stability is secured, and all the adjustments are conveniently placed. The general plan is seen in the illustration.

The Photo-micrographic Microscope G.C.F. made by Carl Zeiss (Fig. 9) is a fine example of an instrument of modern design.

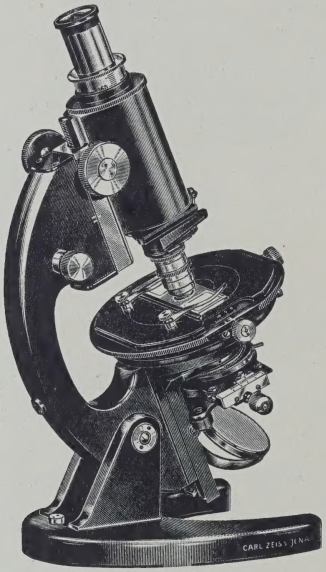

Fig. 9.—Photo-micrographic Microscope G.C.F. (Carl Zeiss.)

The limb is in one piece and carries body-tube stage and sub-stage. The body-tube is large and will accommodate long-focus objectives of the Planar type. The fine adjustment is accurate and positive in its motion. The mechanical stage has a small range but it is easy by its use to centre an object accurately because of its slow motion. The illustration shows clearly enough its main points in design. In common with all Zeiss products this instrument is beautifully made and finished.

The Large Research Microscope by E. Leitz is a fine example of a typical Continental microscope. It has all necessary adjustments except a centring sub-stage. It can be supplied with either a circular rotating stage or one which is square and fixed. The fine adjustment is on the ball-bearing principle which Messrs. Leitz have designed, and which is very accurate in use. The most important point about a fine adjustment, particularly for photo-micrography, is that it shall not only respond well but that it shall stop at any desired point with certainty. It is claimed for the Leitz fine adjustment that these conditions are fully satisfied under all working conditions. The illustration indicates its main features.

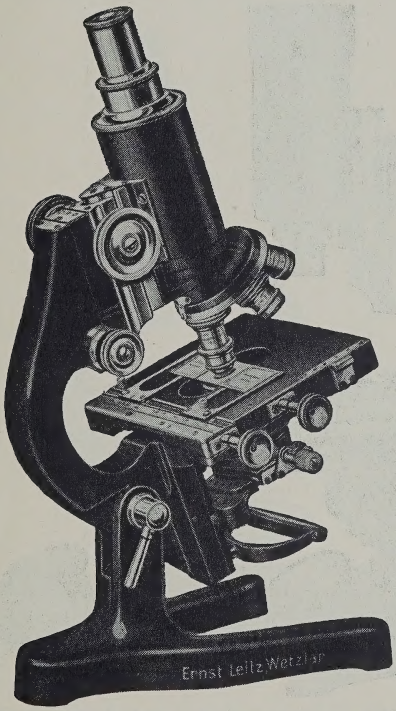

Fig. 10.—Large Research Microscope. (Leitz.)

Fig. 11 shows another type of Leitz microscope with the optical parts removed to allow for the use of low-power objectives or short-focus photographic lenses. Suitable microscopes are also made by Messrs. Reichert of Vienna and Busch of Rathenau, but reference must be made to their catalogues for particulars.

Other recent productions of English manufacture are those of Cooke, Troughton & Simms of York, Charles Hearson & Co. and Charles Perry, both of London. All of these are similar in that they are of simpler design than the foregoing although they embody all essential movements. The illustration (Fig. 12) indicates the main points. In each case mechanical stages are provided or are attachable. None of them have centring sub-stages although these too can be fitted at some additional cost. Each one is a good example of modern machine-tool production and is quite suitable for all ordinary purposes.

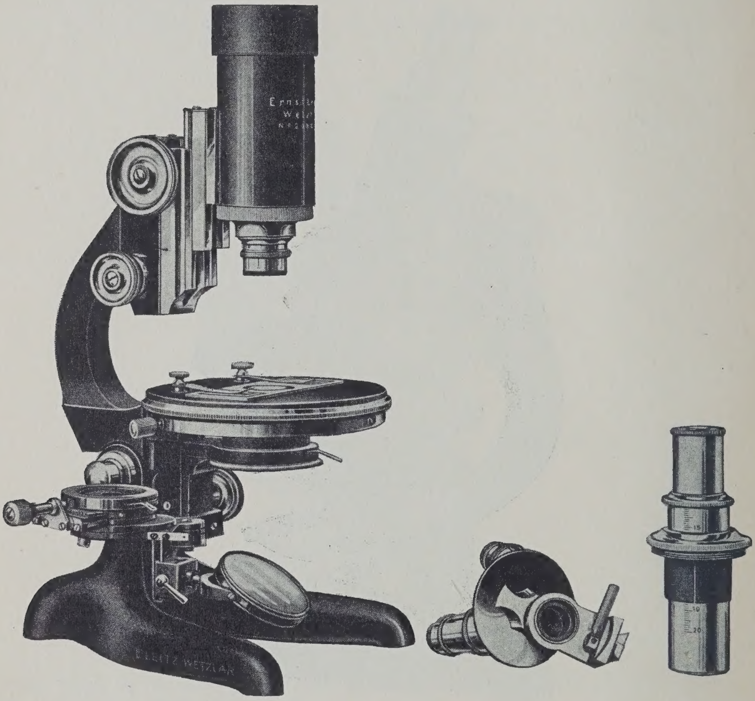

Fig. 11.—Photo-micrographic Microscope. (Leitz).

In the selection of a microscope for any purpose the following points should be considered and are indeed essential if good work is expected. It might be thought that all the possible defects mentioned would have been eliminated by the makers. Experience has shown, however, that this is not always the case, so that it is a reasonable precaution to give some attention to the mechanical construction of the instrument.

1. The microscope should be truly vertical when not inclined, and should be truly horizontal when tilted to its farthest limit.

2. It should be firm and stable in all positions.

3. When the microscope is in the vertical position its stage and its sub-stage condenser carrier should both be parallel to the table on which it stands.

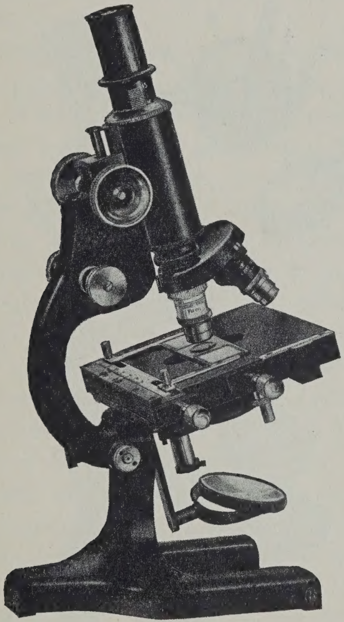

Fig. 12.—Research Microscope. (Cooke, Troughton & Simms.)

4. The stage should be at right angles to the optic axis of the body-tube.

5. The oculars should fit easily into the body-tube but should not be loose. A loose-fitting ocular will cause some change in the position of the centre of the field when the microscope is inclined.

6. The objective screw should be an easy fit to ensure that when the objective is screwed in it shall be true in its seating.

7. The centre of the mirror should be opposite the centre of the body-tube. This condition is frequently departed from.

8. Reference has already been made to both coarse and fine adjustments. The important point is that these movements should be smooth and free ; the control should be such that movement ceases directly the controlling milled heads are released. This applies to every movement in the microscope.

A method of high accuracy for testing microscope stands and fine adjustments has been devised for use at the National Institute for Medical Research and is made by Messrs. Adam Hilger, Ltd. Essentially it is an interferometer, one silvered plate of which is supported on the stage and the other is screwed into the place usually occupied by the objective. Its construction and method of use will be understood by reference to the illustration herewith (Fig. 13).

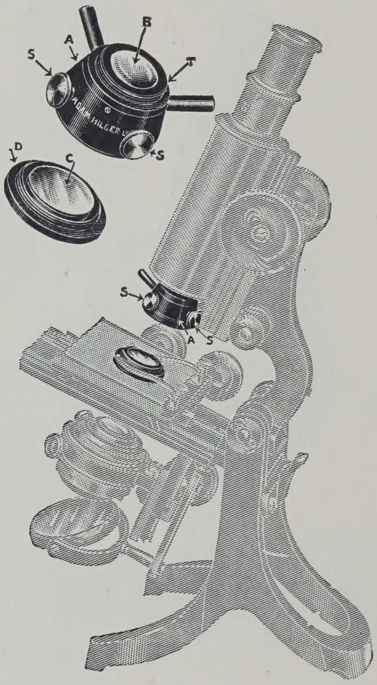

Fig. 13.

Externally it consists of a conical box A, closed by a lens B on the top, and by a silvered glass plate C below. The inner optical parts and surfaces are therefore securely protected from damage and dust. This is important as the instrument thus assembled contains two mirrors, each coated with a thin layer of silver which should not be touched.

In use, the cell D carrying the glass plate C is unscrewed and placed on the stage of the microscope, resting for that purpose on a microscope slide. The box itself is screwed by the thread T in place of the microscope objective. Before attempting any adjustment the box A should be gently lowered by the microscope focussing motion until it touches the cell D, and then raised about 1 to 2 mm.

Light from an Osglim lamp or mercury vapour lamp is reflected upwards by the plane mirror, the condenser of the microscope being removed. With an eye-piece in the microscope, an image of the light source will be seen. The image of the light source will be blurred by multiplication, but by adjustment of the screws S, S, these can all be made to overlap, giving one clear image which can be focussed by using the microscope draw-tube.

The concave mirror should now be put in position, and immediately in the field of view will be seen a number of concentric interference rings; these rings, focussed by the draw-tube of the microscope, may be made perfectly clear by very minute adjustments of the two screws S, S. The rings are formed by interference between the silvered plate which rests on the microscope stage and a similar plate within the conical box, and these rings appear sharply when the two plates are precisely parallel. The purpose of the two screws S, S is to effect perfect parallelism between the two plates. Except in very exceptional circumstances (an especially vibrationless floor, or at times of the day when traffic, etc., is absent) these rings will be seen in constant agitation, and the amount of their movement measured in terms of the distance from one ring to the next is a true measure of the detriment to the image which will result in the use of the microscope.

The rings can be caused to open or close by means of the fine adjustment of the microscope; when one ring increases in diameter so as to replace the next larger ring, one knows that the distance between the two plates, and therefore the distance moved through by the microscope body relative to the stage, has been increased by one hundred-thousandth (one four-thousandth of a millimetre) of an inch very nearly. Since the tenth of this interval can readily be seen by the eye, it will be noted that movements as small as a millionth of an inch can be detected.

The movements which will be injurious to the image are as follows :—

F.	N.A.	Movement of Rings. For Wavelength 5500 A.	For Wavelength 2750 A.
2 mm.	0·75	1·3	0·65
,,	1·50	0·3	0·15
4 mm.	0·6	2·0	1·0
,,	1·20	0·5	0·25

It will be seen that this apparatus affords a valuable check on the movements; not only does it enable backlash to be measured precisely, but it will also enable one to tell the true value of the divisions on the drum-head of the fine adjustment.

It may be pointed out that not only does this apparatus perform the valuable services described above, but it forms a very simple means of demonstrating the principle of interferometry since practically every laboratory possesses a microscope.

CHAPTER III

THE OPTICAL EQUIPMENT: OBJECTIVES AND OCULARS

THE most important part of the apparatus required for photo-micrography is the optical system. It may conveniently be divided and treated under four headings :—

1. The objective, or magnifying system.
2. The ocular, or eye-piece.
3. The sub-stage condensing apparatus for illuminating the object.
4. The collecting or condensing lens or lenses.

As a matter of convenience the last two are separated in this manner to distinguish between the actual sub-stage condenser, as fitted to all modern microscopes, and any optical system that may intervene between the source of light and the sub-stage condenser for the purpose of concentrating or diffusing the light. The latter, which are of greater importance in photo-micrography than in visual work, will be referred to throughout as the collecting-lens or lenses.

The Objective.—It does not come within the scope of this work to deal with the theory of microscopic optics, but an effort will be made to give some simple directions so that a microscopist of limited experience may be able to determine whether the objectives he already has, or may contemplate purchasing, are satisfactory for his purpose. The novice must of necessity rely on the judgment of others, depending on those from whom the apparatus is purchased, or preferably seeking the assistance of a friendly expert.

The primary consideration in an objective is focal length as it is mainly on this factor that magnification depends. Magnification is greatest when the focal length of the objective is short: it can be altered within limits by change of ocular or of tube-length. Alteration of tube-length is only permissible for certain definite reasons to be referred to later, but oculars vary in focal length as do objectives, so that they are an important factor in determining the final magnification obtained.

There are two well-defined types of microscope objectives available—achromatic and apochromatic—the difference between them depending on their degree of correction for certain aberrations inherent in an objective. Each type may again be subdivided into low- and high-power lenses. The lower powers are always what are known as dry lenses, and with them a magnification of not more than five hundred diameters will be obtainable. The high powers are chiefly what are known as immersion lenses; that is, between the object and the front lens of the objective an immersion fluid—sometimes water, but usually cedar-wood oil—is used; or, in the case of some lenses of very high aperture, monobromide of naphthaline. The object of the immersion fluid is to enable a wider beam of light to be utilised by the objective. Owing to cedar-wood oil having a refractive index approximately the same as glass, the system becomes what is known as 'homogeneous'; that is, assuming the object to be mounted in contact with the cover-glass, or in a medium of nearly the same refractivity as glass, little or no refraction of the light occurs from its leaving the object until it passes into the objective. Consequently, more light is transmitted to the objective, and a beam of wider angle can be utilised.

Fig. 14 represents diagrammatically a section through the cover-glass covering any object and the front lens of an objective. The left half represents the conditions under which an oil-immersion objective functions, and the right half shows the air-gap between cover-glass and objective which exists with dry lenses.

It will be seen that the ray AB undergoes refraction at B when entering the cover-glass, being refracted towards the normal BN, and, on leaving the glass, is again refracted in the direction DA′ parallel to AB. The consequence is that the ray AB issues from glass into air as DA′, and does not enter the front lens of the objective. The ray CBC′ is the ray farthest from the normal that enters the lens. The condition of affairs when oil is interposed is seen on the other side, where the ray

EB is again refracted by the cover-glass; but, as the oil is of practically the same refractive index as the glass, the ray continues in the same straight line in the direction BE′ and enters the lens, thus taking part in the formation of the image. A ray, therefore, such as FB, which is very much more oblique and which would take the direction BF′ on refraction through the glass, would just enter the lens. It is evident, therefore, that with an oil-immersion objective more light enters, and light also of a very much wider angle, than is the case with a dry lens.

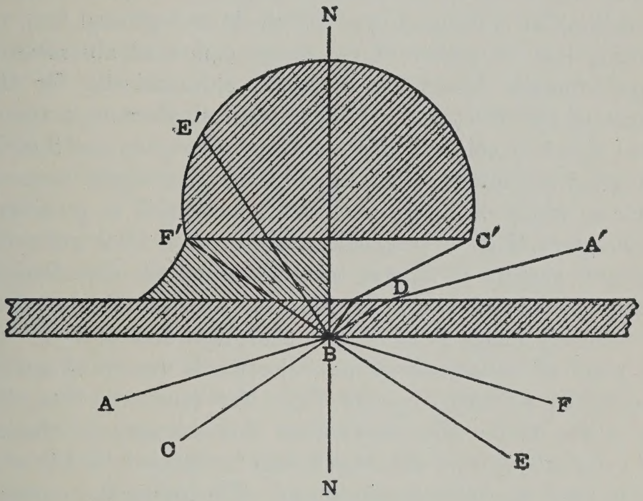

Fig. 14.—Diagram showing Effect of Oil Immersion as compared with a Dry Lens.

The difference between achromatic and apochromatic objectives is a fundamental one, although it must be admitted that the dividing-line is becoming ever finer. Unfortunately some lenses described and sold as apochromatic are not entitled to be so designated. The term ‘ semi-apochromatic ’ has also been introduced, and is sometimes used to describe improved achromatic lenses, particularly those in which fluorite is used as one of the components of the optical system. Although the lenses are an improvement on the ordinary type they are not equal to apochromatic lenses in perfection of correction.

The difference between these two types of lenses is dependent on the degree of correction for chromatic and spherical aberration. In achromatic lenses chromatic correction is for two colours of the spectrum, whereas the correction for spherical aberration is for one colour. Thus there is always an outstanding portion of the spectrum with which the lens is not able to deal, and which, therefore, is not utilised in the formation of the perfect image. It becomes evident either as a more or less visible fringe of colour around the image of the object (chromatic aberration), or is diffused over the whole as a general haze with resulting loss of quality of the image (spherical aberration).

Apochromatic lenses are corrected chromatically for three colours of the spectrum, and for spherical aberration are corrected for two colours. For photographic purposes this is a very great advantage; but, in addition, their higher numerical aperture, which owing to their construction it is possible to attain, gives them a proportionately greater resolving power. A greater amount of light is transmitted by the objective, and consequently in photography the exposures are shortened and the resulting image is of greater sharpness and purity.

A point of importance in an objective is numerical aperture—ordinarily referred to as its N.A. The aperture of an objective is the factor that determines the obliquity of the rays that, emanating from the object, can be utilised by the objective to form the microscopic image. The higher the numerical aperture the larger the cone of rays that enters the objective. It therefore affects the brightness of the image, the larger the aperture the greater the light-gathering power, a point of more importance in photo-micrography than in the observation of a visual image. N.A. is of primary importance when the utmost resolution is required. The possibility of reproducing a correct image of every point in an object is dependent upon the numerical aperture of the objective used and on the wave-length of the light used as an illuminant. A point in an object which in size is at the limit of resolution is represented in the image not by a mathematical point but by a disc of definite dimensions, usually referred to as a ‘diffraction disc.’ The diameter of this disc becomes greater as numerical aperture is decreased. Since all microscopic objects may be regarded as being made up of a number of points or similar elements of structure the satisfactory separation of these, especially when they are close together, is the most important factor in the formation of an image and is the one in which numerical aperture with its resulting resolution plays the largest part. Until comparatively recently a quality known as penetration, or what is usually referred to in photographic lenses as depth of focus, was regarded as an advantage, and was sometimes referred to as an especially good point when a lens was to be used for photo-micrographic work. This is no longer the case, since the penetration of a microscope objective decreases as numerical aperture is increased. Some apparent increase in depth of focus may be obtained by reducing the aperture of the sub-stage condenser, the reduction being effected by closing the sub-stage iris-diaphragm. A narrower cone of rays, therefore, enters the objective, causing it to work at a smaller numerical aperture. This is a harmful proceeding if carried to excess, and such a method is not advisable for the purpose of obtaining penetration except under very special conditions. For some classes of work lenses of small aperture are still used—particularly where it is desired only to obtain a general idea of the appearance of an object of considerable thickness; but for exact work, whether visual or photographic, lenses of the highest obtainable aperture are to be preferred. In visual work some accommodation usually results from the continual raising and lowering of the objective by the fine adjustment, so that a concrete notion of the appearance of an object in depth is obtained, but in photography this is not so.

Although it has been pointed out that the quality of penetration is in general not desirable in microscope objectives, it does sometimes happen in practice that the delineation of structure in depth is useful. The method of obtaining this penetration by reducing the aperture of the sub-stage condenser is open to objections, and a more satisfactory way is to reduce the aperture of the objective itself. With low-power lenses this may be effected by having immediately behind the objective, in the position where it screws into the microscope tube, an iris-diaphragm which may be contracted at will. With high-power lenses, however, this is not a practical method, as it is not possible to get a diaphragm sufficiently close to the posterior combination of the objective effectively to stop it down. Microscope makers now construct small metal funnels that drop into the back of the objective and have an opening which is nearly in contact with the back lens. These contrivances are made so that a definite reduction in aperture is ensured, and one of these stops has to be obtained for each aperture. With an oil-immersion lens therefore of, say, an aperture of 1·40, a stop that would reduce it to 1·20 or 1·10 would probably meet all ordinary requirements. A simple diagram (Fig. 15) may explain the effect in increasing penetration by stopping down the objective.

Suppose the line FF to represent one plane of a thick object, and that the object extends in depth in successive layers in the direction of the point A. The point C may therefore be only a little below the focal plane of the objective; and if the illumination is by a narrow cone of light BB, the appearance of the point, viewed in relation to the line FF, will be less spread, and approximately in better focus than if it were illuminated by a wide cone included within the angle DD. The consequence is that if there are in the object a succession of points lying just below the line FF, these will appear less diffused with a narrow angle than if they are viewed with a wide cone of light. It is clear also that the apparent difference between the size of the image-points in depth will be less as the angle of illumination becomes narrower. Thus with a thick specimen a cone of narrow angle will give images

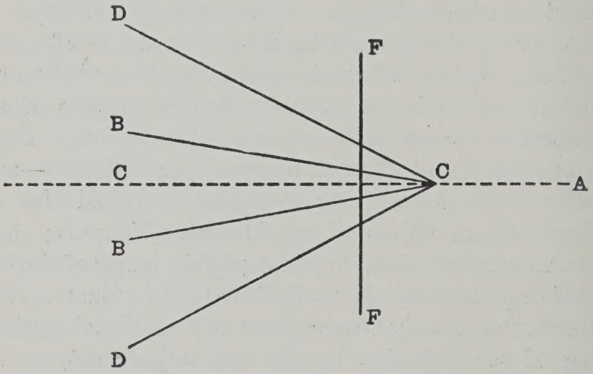

FIG. 15.—Increased Penetration due to stopping-down of Objective.

—assuming them to be a series of points—that vary less in size than they would if the cone were wide; they will not, however, be such well-defined images, even when exactly in the focus of the objective, as would be obtained by utilising a higher numerical aperture.

The evil of carrying the reduction of aperture too far is that diffraction fringes soon begin to appear around each element of structure, and should these diffraction fringes be sufficiently evident and large enough to overlap, a general want of definition results.

This ability to observe overlying elements of structure is generally of doubtful advantage, and this is certainly true when exact delineation is required. A compromise is often desirable when a somewhat diagrammatic representation of an object is needed, and this is the only justification for any procedure that enables such images to be obtained. When exact structure is to be represented in a photograph the highest practicable numerical aperture should be used, so that one plane is in accurate focus and all other planes, either above or below, are quite out of focus. Whilst reduction of aperture results in some apparent penetration, the converse is also true that a large aperture shows up one plane as perfectly as possible by rendering less evident the effect of other out-of-focus images. This is a practical point of great importance, and it is only by studying the effect of variation of aperture on the appearance of the image that full use can be made of this ability to eliminate undesirable structural appearances. No description is sufficient to enable a worker to understand the effect obtainable; he must observe for himself. Further, no two objects are the same in this respect, the object itself is an important factor in deciding what proportion of the objective aperture it is possible to use. Elements of structure of high refractivity themselves increase the effective N.A of an objective even when some reduction of the illuminating cone of light is necessary. This is seen in the photography of diatoms where the illuminating cone may be small, although the full effective aperture of the objective is often brought into use. To recapitulate, therefore, always use the largest possible cone of light and thereby obtain the best resolution in one plane. This point will again be referred to at a later stage.

Another factor influencing penetration is the density of the medium in which the object is mounted; a more highly refracting medium giving greater penetration than one of less refractivity. It follows that an object mounted in Canada balsam would be better suited, on general grounds, for photographic purposes than one mounted dry. Diatoms, or similar objects, which are mounted in media of high refractive index —such as realgar or styrax—might be expected to give somewhat better results in this respect, other things being equal, than would be the case if they were mounted in less dense media.

The total depth of focus is the sum of the accommodation depth—a property of the human eye—and what may be termed the diffusion or focal depth—a property of the objective. It is the latter that is alone operative in photo-micrography, but it is less than the accommodation depth. As examples it may be assumed that :—

A two-third-inch or 16-mm. objective with an N.A. of 0·30 having an accommodation depth of about 1/25th mm. would have a diffusion depth of 1/200th mm. approximately.

A one-twelfth-inch or 2-mm. objective of 1·40 N.A. would have an accommodation depth of about 1/1600th mm. and a diffusion depth of rather less than 1/7000th mm. It is assumed in both cases that the object is mounted in a medium homogeneous with the cover-glass.

For all practical purposes it may be assumed that penetration varies inversely as the N.A. and directly as the mean wave-length of the illuminant. It is only the former factor that need be considered under most ordinary circumstances.

Hence, for photography, the principle is a sound one to use an objective of the lowest power and the highest N.A. that will show the desired detail.

Metallurgical specimens illuminated by reflected light are mounted without a cover-glass, and therefore without any mounting medium. This is to obviate the extra reflection that would occur from the surface of the cover-glass, and with such preparations better results are obtained by the use of oil-immersion objectives than with dry ones. The immersion fluid is in this case placed directly in contact with the specimen, and so the conditions again occur in which the greatest penetration is obtained.

Another quality of an objective which is of importance, particularly in low-power lenses, is what is known as flatness of field. With no objective is the whole field of view in proper focus at one time—at least, when a moderate-power eye-piece is used. With some oculars this does not appear to be true, but this is because the diaphragm in the ocular restricts the size of the visible field. It would appear that increase of numerical aperture has tended to decrease flatness of field, as a small field is characteristic of objectives of large aperture, especially the high-power ones. It is at times convenient to have a lens that covers a large field, even if the definition is not so perfect as might otherwise be desirable, but usually it will be found that lenses of good quality have sufficient covering-power for any ordinary requirement. Flatness of field should not be regarded as a point of first importance; a lens with this quality should only be selected when all other requirements have been fulfilled. It is a quality easy to determine, since with any flat object it can at once be seen where definition begins to fall off, and what proportion of the field is actually in focus at one time.

With a good objective, although the whole field of view may not be in focus at one time, any alteration of the fine adjustment should result in successive zones of the field coming into focus, and the quality of the image should be nearly as good at the margin of the field as it is at its centre.

On the numerical aperture of an objective depends its power of making visible and separating fine details in an object, and it is dependent upon the angle of the cone of light collected by the objective from each point in the object. It must not be confused with angular aperture, or the method of indicating the aperture of photographic lenses, as these are dependent on the total angle of the field of view. Numerical aperture is purely a function of the objective, the ocular only amplifying the image, and adding nothing to the resolving power of the optical combination.

The term ‘resolving power’ means the power of resolving or properly delineating fine regular structure, and must not be confused with magnifying power or any arrangement by which mere amplification is obtained. Neither does it indicate the limit of visibility; small isolated objects, or the components of irregular structure, may be seen that are considerably smaller than the distance between the components of a regular structure—such, for instance, as occurs in a diatom or a mechanically ruled grating. Consequently it is desirable to use lenses of the highest obtainable aperture, but of the longest focus—in other words, the lowest power lens that will show what is required. It is seldom necessary to master the various methods of measuring the N.A. of objectives, as this is usually marked on the lens mount. Good makers now take care that the aperture which they claim is justified; but as it may happen that such methods of measurement are required, the best known and most reliable of these are described in the Appendix. A very complete table is published with the *Journal of the Royal Microscopical Society*, setting out the number of lines per inch that may be resolved with a given aperture under given conditions of illumination, and reference should be made to this for such information.

Where the question of cost is of no consequence, a series of apochromatic lenses will place the photo-micrographer in possession of the best objectives, with the highest N.A., that can at present be obtained. On the other hand, achromatic objectives are being so improved that no one need despair of producing work of a high class by their aid, especially if care is taken, by methods to be explained later, to ensure that any outstanding colour for which they are not corrected is eliminated.

Objectives are described in terms that represent their approximate initial magnifying power without an eye-piece. This description does not of necessity indicate the distance between the front of the lens and the object; although there is, speaking broadly, some relation between this distance and the power of the objective.

Owing to the desire for higher aperture lenses, the indication of focal length has become somewhat loose; for it is easier both optically and mechanically to produce a lens of high aperture and proportionately high magnifying power than the reverse. The consequence is that a lens described as a one-inch objective, with an aperture that would be a high one for such a focal length, is in some cases little more than, say, three-quarters of an inch. This is shown by Fig. 16, where *a* was photographed with a one-sixth-inch achromatic objective, and *b* with a 4-mm. apochromat; it will be seen that the achromatic lens gives a distinctly higher magnification although the assigned focal lengths are practically the same.

If it is desired to know the exact magnification of an object, either visually or photographically, it will be necessary to determine it by the aid of a micrometer. The methods to be employed for such determinations will be fully described later. (See Chap. VIII.)

For exhaustive methods of testing and choosing objectives, the reader must refer to books on microscopy,[1] but it may be convenient if some simple tests are indicated, so that in purchasing lenses reliance will not entirely be placed on the seller, and a worker will derive some extra satisfaction from discerning for himself the differences between the lenses. It is advisable, however, to purchase only from makers of repute, and in that case no difficulty will be encountered in obtaining lenses that are satisfactory.

There are certain well-recognised tests for objectives, and it is often asserted as a recommendation that a lens will resolve the details of a particular test-object. This is regarded as a proof of its excellence, but it is by no means a certain or even a satisfactory method. In any case, the test-object should be one well known to the observer; for example, in a *Podura* scale, or in certain diatoms, there is so much variation in the objects themselves that they often afford little information as to the quality of the objective.

But where an object is known, an expert observer can tell, by comparing the images given by different objectives, what the qualities of the latter really are. For comparative purposes therefore such test-objects are of undoubted value.

For low-power objectives it is probable that no better test exists than an object so illuminated that it shows up bright on a dark ground—i.e. by what is ordinarily known as 'dark-ground illumination.' This condition may be obtained by putting a stop into the sub-stage condenser to cut out the central rays and only allow the peripheral rays to pass; these impinge on to the object at a considerable angle, so that no rays enter the objective unless they are refracted or reflected by the object. Such stops for oblique illumination are usually supplied with microscopes, and are adapted for use with the sub-stage condenser. With powers of one inch or less, this is perhaps as good a test as can be used, and with it the image should appear perfectly sharp and keen, with no haziness or evidence of diffused light at the edges. A suitable object for this test is a section of an *Echinus* spine, or an unstained botanical section. For tests with axial illumination, the well-known object, the blow-fly's tongue, will be found suitable; the hairs on its outer edge should show sharp and black, and the points of each hair should be seen perfectly to the extremity. There should be no indication of nebulosity or duplication of the edges of the image, but care must be taken to obtain the correct tube-length for each lens.

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[1] See Carpenter's *Microscope and its Revelations* (J. & A. Churchill, London) or Spitta's *Microscopy* (John Murray, London). A more recent work is *The Microscope*, by Conrad Beck, published in two parts, by R. &. J. Beck, Ltd.

With any objective very useful information as to its defining power can be obtained by using with it a high-power eyepiece—say, one magnifying fifteen times. It will be a good achromatic lens that, with any ordinary test-object, will stand this test without loss of definition. One in which the image remains sharp and clear with an eye-piece magnifying twelve times is, speaking broadly, a good lens.

With apochromatic objectives this does not apply, for in low and moderate powers these will not break down or show any evidence of deterioration of the image if an eye-piece of the highest available power is used.

With dry lenses of less focal length than one inch, the *Podura* scale, or any of the coarser diatoms, will answer for purposes of testing. In any case the scale or diatom must be selected so that it has well-defined markings, and these should always show a clean bright image, with no nebulosity or haziness at the edge. What are known as the 'exclamation marks' on the *Podura* scale should show up sharply, with their extremities going off to a sharp point.

For oil immersion objectives, one of the finer diatoms—such as *Pleurosigma angulatum*, or *Amphipleura pellucida*—is the recognised test; and certainly for objectives of high aperture either may, in the hands of an expert, fulfil every requirement.

If, however, such diatoms are employed, the differences in appearance with varying methods of illumination are so marked that it is impossible for an inexperienced microscopist to infer anything from them. The writers are of the opinion that for an ordinary test of an oil immersion objective, a stained preparation of bacteria is to be preferred. With this, in conjunction with the method already mentioned for testing the effect of high-power oculars, a great deal can be learnt of the qualities of an objective. Preparations should be chosen that are sharply stained, and it is advisable to select one (such as *Bacillus tuberculosis*) which has a segmented or beaded appearance ; or some sporing organism (such as *Bacillus anthracis*) since in these it is easier to observe the more subtle differences in the quality of the image.

For testing for chromatic and spherical correction, as well as for flatness of field, there is no better object with either high or low powers than the Abbe test-plate. This, further, has the advantage that where thickness of cover-glass is of great importance (as it unquestionably is with any high-power dry lenses), the most perfect correction that corresponds to a particular thickness can be estimated with accuracy. The test-plate also has an educational value, as it enables an inexperienced worker to observe the effect of change of tube-length with any given thickness of cover-glass. In addition the changes in the image due to different thickness of cover-glass with any given tube-length may be seen, and sufficient experience can be gained to make the necessary corrections by observation of the image. No one has any right to regard himself as a microscopist until this can be carried out with certainty. The test-plate consists of six discs of thin microscopic cover-glass of accurately determined thickness (0·09 mm. to 0·24 mm.) cemented side by side. Each of these discs is silvered on its lower surface, and has a series of lines ruled on it, so that it is in effect a coarse grating of alternating silvered lines and clear spaces. The lines are usually not quite opaque, but transmit sufficient light to enable the grain of the silver deposit to be seen. On examination under the microscope with a low power, therefore, a large number of these lines come into the field, but with high powers possibly only two or three lines will be shown : in either case the object is so thin and each line has such a number of prominences on either edge that it affords an extremely valuable, and comparatively easy, method of determining some of the qualities of the objective under examination.

When the image is exactly in focus under axial illumination the edges of the lines should appear perfectly sharp with no apparent doubling; neither should there be any but the very slightest indication of colour fringes with achromatic lenses.

The critical test is to use oblique light, obtained by putting an excentric stop beneath the condenser, and the light should fall in a direction at right angles to that of the lines ruled on the test-plate. Each objective should be tested first by oblique, and then by direct axial light, and on changing from one method to the other no alteration in the focus of the objective should be necessary—that is, the object having been focussed by oblique light, its image should appear to be equally well defined when illuminated by axial light. This test indicates the degree of correction of the lens for spherical aberration.

To test for chromatic correction the same methods of illumination should be arranged. An achromatic lens should show only narrow fringes of colour on each side of the lines on the test-plate, and these should be the complementary colours of the secondary spectrum, on one side greenish, and on the other a reddish violet. With apochromatic objectives of the highest quality there is little or no evidence of any coloured margin of the lines under any conditions of illumination.

In working with a test-plate the light used should be fairly bright and as white as possible. It is also necessary to have one or two oculars of high power.

It is probably understood that the value of an objective depends not on its magnifying power, but on its ability to resolve fine details, and this is mainly a function of its aperture. In testing objectives for ordinary work, it is not only necessary to determine the resolving power, but more especially to find out the quality of the image, and the power which the objective has of forming an image. The tests already mentioned, while in no way entitled to be regarded as exhaustive, may assist an ordinary observer in gaining some information as to the extent to which his objectives have this property. The illustrations (Fig. 16, *a*, *b*, *c*, *d*) will give some idea of the appearances that may be expected when using certain objectives in combination with a particular ocular. They are all photographs of the Abbe test-plate. Fig. 16 *a* is taken with a one-sixth-inch achromatic objective of undoubtedly good quality, in combination with a No. 2 projection ocular. For comparison, Fig. 16 *b* has been taken under exactly similar conditions, but a 4-mm. apochromatic has been used instead of the one-sixth-inch achromatic. The difference between the images, both in definition and general brilliancy, is at once apparent, and will give an idea of the appearance that may be expected when observations are made for the purpose of determining the quality of objectives.

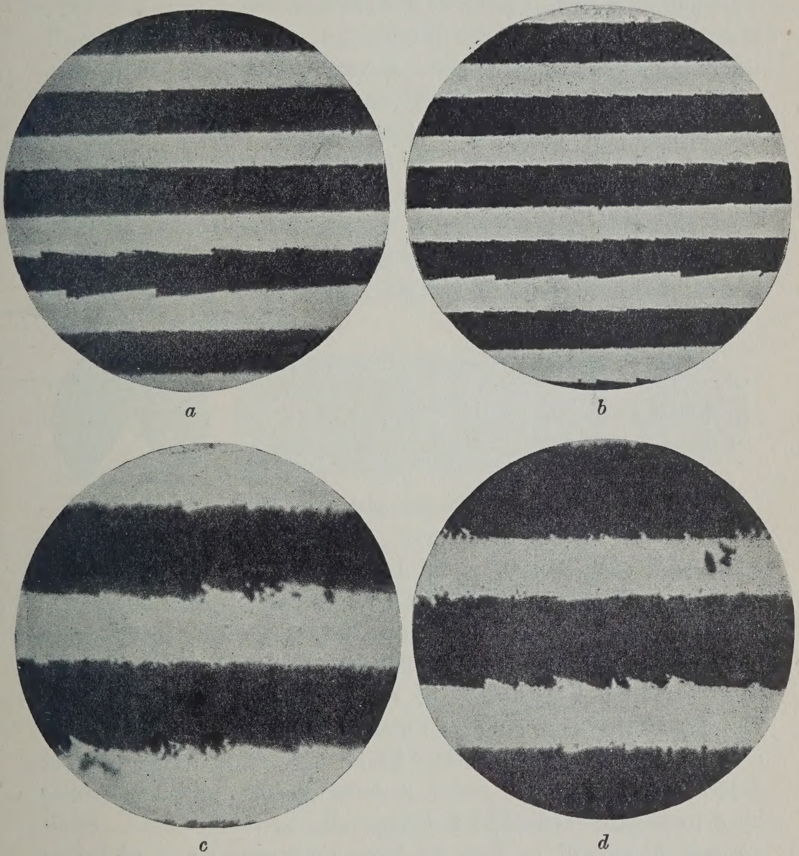

a *b* *c* *d*

FIG. 16.—Photo-micrographs of Abbe Test-plate.

An improved form of test-plate is now made by Messrs. Zeiss, although it is doubtful whether a novice will find it so easy to use as the earlier form. In this the silver lines are deposited on a thin glass wedge, which varies in thickness in the direction of the length of the slide on which it is mounted (Fig. 17). The wedge is in the form of a cover-glass and increases regularly from about 0·10 mm. to 0·25 mm., thus including all ordinary cover-glass thicknesses. A very simple but useful test-plate can be improvised by anyone familiar with the ordinary procedure in mounting microscopic objects. A few cover-glasses must be obtained of carefully calibrated thickness, and they should vary from about 0·12 mm. to 0·25 mm. in regular steps. They should be of small size, so that six at least can be mounted on a 3 × 1-inch glass slip. On each one a trace of very dilute Canada balsam should be smeared, and then a piece of gold or silver leaf, such as is used by decorators, evenly pressed down so that the leaf is in close contact with the cover-glass surface. These cover-glasses are then mounted on the slide in the ordinary way, the thickness being marked against each or alternatively, numbered according to an arranged plan. It will be found that there are sufficient breaks or uneven places in these metal films to enable testing to be carried out successfully. Alternatively a similar series of cover-glasses may be silvered by a method described in the Appendix to this volume. Such silvered films will be sure to have numerous small pin-holes and streaks in them, which again can be used for testing purposes.

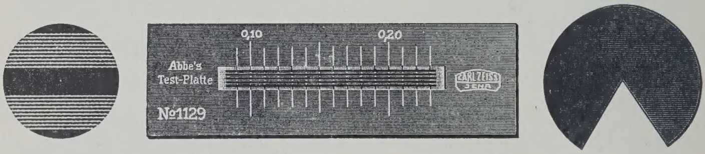


Fig. 17.—Abbe Test-plate. (C. Zeiss.)

Figs. 16 *c* and 16 *d* are taken under similar conditions, except that projection-ocular 4, in conjunction with a somewhat greater camera extension, has been used. Fig. 16 *c* is taken with the achromatic, and Fig. 16 *d* with the apochromatic objective. Here again the difference can be seen immediately, and owing to the higher power ocular employed is accentuated.

Another point worthy of note in these figures is the difference in centration that occurs with different objectives. The two objectives were screwed into the same sliding objective-changer and were then placed on the microscope: when Figs. 16 *a* and *b* and also *c* and *d* are compared, it will be seen that a totally different field of view has been included by each objective. Thus the difference in centration is—in this particular case—at least equal to the area of the entire field of view. This is by no means an exaggerated instance, and is a state of affairs that may be expected when any considerable number of objectives are being used in succession. It demonstrates the necessity of using a method of changing the objectives which will admit of their correct centration one to another and to the optical axis of the microscope.

A good selection of objectives for photo-micrographic work would be the following:—

1. A Planar or micro-Summar, or a lens of similar construction (preferably one of rather long focus, either the 75-mm. or 100-mm.), for photographing large objects or sections.
2. A 1½- or 1-inch achromat.
3. A two-thirds-inch or 16-mm. apochromat.
4. A one-third-inch or 8-mm. apochromat.
5. A one-twelfth-inch oil immersion achromat, and either a 3-mm. or 2-mm. (N.A. 1·30 or 1·40) apochromat. The 3-mm. apochromat should be chosen in preference to a 2-mm. for general work. Its greater focal length gives it a longer working distance, and it may be used with the highest power oculars.

The possessor of either series of these lenses would have a complete battery, and would be in a position to deal with any class of photo-micrographic work. The apochromats should be chosen if possible; but, as already stated, it need not be regarded as an insuperable obstacle to achieving fine results if these cannot be obtained.

When such a complete outfit cannot be procured, a one-inch and one-sixth-inch lens will be most useful for general work. If bacteriological work is to be attempted, little can be done without the addition of a one-twelfth-inch oil immersion objective; and where it is intended only to do the latter class of work, an oil immersion objective, with a two-thirds-inch in addition, could be made to answer all ordinary requirements.

It would certainly be better to acquire, say, the 16-mm. and the 3-mm. apochromats, as with these two lenses, and with suitable combinations of ocular and camera extension, almost any degree of magnification could be obtained, except of course if large objects are to be photographed. For the latter any good-quality photographic lens—perferably an anastigmat—will be found satisfactory.

Apochromatic objectives are to be preferred to achromatic ones as there is more perfect union of all the rays transmitted by the objective. This becomes more evident in a photograph as the least visible rays are the most actinic, the deep blues and violets particularly so. Most makers now produce apochromatic objectives, and recently some have improved on the quality of pre-war lenses. R. & J. Beck, Ltd., have specialised in this direction and their latest products are probably unequalled, particularly those of 8-mm. and 16-mm. focal lengths. Post-war efforts have been mainly in the direction of raising the standard of existing types of objective, but in one direction a change is evident, due in large part to the greater use of dark-ground illumination. This has resulted in the production of oil-immersion objectives of greater focal length than those formerly used. Both Zeiss and Leitz now make one-seventh-inch focus lenses, of N.A. 0·95, for such a purpose, and further reference to these will be made when dealing with the subject of dark-ground illumination. Other immersion lenses of this type and of first-rate quality are those of one-eighth-inch focus made by R. & J. Beck, Ltd., J. Swift & Son and W. Watson & Sons. These objectives do in large part take the place of the one-twelfth-inch oil immersion, and they may also completely eliminate the one-sixth-inch or any similar dry lens. The latter is really the most difficult of all to use and is rarely satisfactory for photography.

The initial magnification obtainable with various typical objectives, and also with the same lenses in conjunction with certain specified oculars, are indicated in Tables I and II.

It must be understood that these magnifications apply only when the conditions indicated are exactly fulfilled.

With most microscopes an appliance, called a nose-piece, for changing the objectives, is now supplied ; the nose-piece may be either double, triple, or quadruple, carrying two, three, or

TABLE I

ACHROMATIC OBJECTIVES

English Designation.	Focal length.	Numerical aperture.	Initial magnifying power.	Magnifying Power.		
				With Eye-piece. Focal lengths.		
				42 mm.	25 mm.	17 mm.
inches.	mm.					
$\frac{1}{2}$	32	0·15	4	25	45	65
$\frac{2}{3}$	16	0·28	10	62	110	155
$\frac{1}{3}$	8	0·54	18·5	115	200	285
$\frac{1}{6}$	4	0·85	40	285	490	690
$\frac{1}{8}$	3	0·95	60	427	735	1,035
$\frac{1}{12}$	2	1·3	87	530	900	1,275

TABLE II

APOCHROMATIC OBJECTIVES

$1\frac{1}{2}$	40	0·16	3	20	34	50
$\frac{2}{3}$	16	0·3	10	62	110	155
$\frac{1}{3}$	8	0·65	18·5	115	200	285
$\frac{1}{6}$	4	0·95	40	285	490	690
$\frac{1}{12}$	2	1·3	83	530	900	1,275

four objectives respectively. It is screwed into the microscope in the position usually occupied by the objective. On it is a rotating plate so arranged that the objectives which it carries can be brought successively into alignment with the axis of the microscope. Such appliances are described and figured in all makers' catalogues and usually form part of the equipment of any ordinary microscope. While they are useful for visual work, they are rarely to be recommended for photographic purposes. It is unusual for a revolving nose-piece to be so accurately constructed that the objectives, when brought into the position of the optical axis, will be equally well centred. The consequence is that if the object is centred with one objective, say a low-power one, and then a high power is brought into use, the object is not in the centre of the field. In some cases the want of centration is so great that the image may be altogether out of the field.

Some makers are giving greater attention to these appliances, and the results obtained are better than formerly; but it is still rare for a triple or a quadruple nose-piece to be so accurately made that all the objectives which it carries are equally well centred.

Another objection to these multiple-changers is that a considerable additional weight—consisting of two, three, or four objectives as well as the changer itself—is thrown on to the body-tube of the microscope; and as this weight is thrown only on one side it is a very real objection in accurate work. A further disadvantage is that it is difficult to ensure that the optical axis of the objective is in exact alignment with the axis of the microscope, and should defective alignment occur with a lens of large aperture it would interfere seriously with its performance. It is better, for photo-micrographic work, to do without such a method of changing; though it may be convenient, it introduces a possibility of error in the resulting photograph.

The sliding objective-changers made by Messrs. Zeiss (Fig. 18) are superior to revolving nose-pieces as they provide means for centring objectives to the optic axis of the microscope and to one another, while the number of objectives that can be in use is unlimited. There are two elements, one the tube-slide and the other the objective-slide. Fig 18 shows the constituent parts of such changers, *a* represents the tube-slide screwed into the microscope body-tube and below it is seen an objective-slide with objective attached; *c* shows the two parts in combination, body-tube and objective being shown in outline only.

The tube-slide is shown in section, except the part bearing the screw thread. The slide is not rigidly connected with this part but may be turned so long as the screw is not tightened up. This admits of the tube-slide being clamped with the plane of the slide action turned either to the front of the microscope or to one side. The objective-slide shows on its right a screw for the purpose of centring the objective in a direction parallel to the slide action, while the screw below it serves for centration at right angles to the slide. The inclination of the slide action causes the objective to rise when being changed.

It is advisable to keep each objective to its respective slide after centration has been effected.

It should be noted that when using either the nose-piece or objective-changer due allowance must be made for these in estimating tube-length.

A simple objective-changer which is not open to the objections urged against those of the double or triple nose-piece type is made by Messrs. W. Watson & Sons. The advantages put forward by the makers are that it is very thin and only increases the tube-length by 12·5 mm.; that it is simple and reliable in use, and as it is made of magnalium it is light, and so adds but little to the weight carried by the microscope tube. The turning of the milled head results in the inversion downwards of a pair of jaws having a screw-thread cut upon them. The objective is placed in the aperture, and directly the milled head is released, the jaws are carried back and engage the threads of the objective. It therefore acts as a permanent attachment to the body-tube of the microscope, and each objective may be inserted without the necessity of screwing in. It does not admit of exact centration of each objective as does the Zeiss changer, but in use it would certainly be better than a multiple nose-piece.

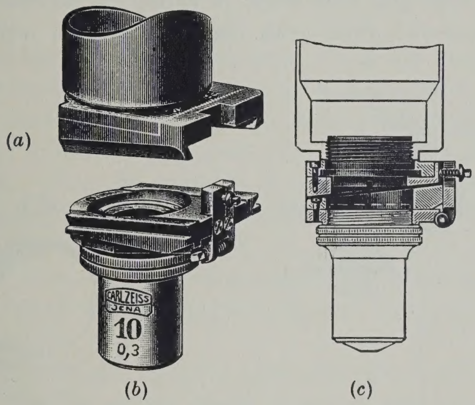


Fig. 18.—Sliding Objective-Changer. (C. Zeiss.)
a, tube-slide; *b*, objective-slide with objective attached; *c*, intersection. (Full size.)

Another appliance for the same purpose is the Sloan Objective-Changer (Fig. 19) made by R. & J. Beck, Ltd.

It consists of an adapter which screws on to the nose of the microscope—this adapter has on one side a sloping projection (A), and on the other a screw clamp (B) with a bevelled nut (C). The adapter is clamped to the nose-piece of the microscope by a screwed ring (D), which is provided with slots, into which a halfpenny will fit for tightening it up.

Loose fittings are supplied, one of which is screwed on to each object glass.

Each fitting has a bevelled gap which fits loosely over the bevelled nut (C) of the adapter and swings round into position. A turn of the milled head (B) forces the fitting against the sloping projection (A) and holds it firmly in position. Each adapter has screwed studs with clamping screws, which form the stops in both directions when the objective is in the correct position. These can be adjusted by means of a spanner supplied for the purpose, so that each lens can be centred with an accuracy that is never possible with a revolving nose-piece.

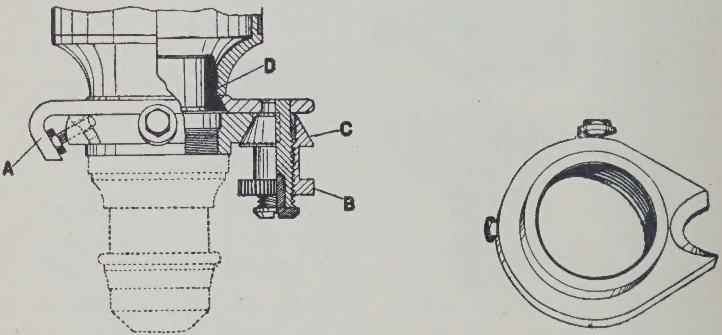


Fig. 19.—The Sloan Objective-Changer. (R. & J. Beck, Ltd.)

The construction of this apparatus is so simple and rigid, having no slides to wear loose, that it remains permanently in adjustment.

The total extra length to the microscope body caused by its use is 10 mm.

A box is supplied to carry objectives with the fittings screwed on ready for use, thus keeping them free from dust.

The Ocular or Eye-piece.—An important optical part, and which in the case of the apochromatic objectives is an essential portion of the magnifying system, is the ocular. Its function is to pick up and transmit to the eye—or to the photographic plate—the image already formed by the objective, always amplifying it to a greater or less extent.

There are many types of oculars, but for microscopic work the two used almost universally are those known as the Huyghenian and the Ramsden. These may again be divided into ordinary and compensating: the latter distinction depending on whether they are for use with achromatic or apochromatic objectives. Both of the first-mentioned types occur in the Zeiss series of compensating oculars—the lower power oculars being of the Huyghenian and the higher power of the Ramsden type.

In referring to ordinary oculars, which are suitable for use with achromatic objectives, some makers have adopted an arbitrary method of description—simply designating them by a letter or number which gives no indication of their amplifying power. They are made in a series of regularly increasing power, and are called by the letters A, B, C, D, E and F, the magnification usually being 6, 9, 12, 18, 21 and 24. This method is however being discontinued by most makers; both the focal length and the magnification are now usually engraved on the mount.

The images formed by achromatic objectives are in themselves complete, and the ocular should cause no subsequent alteration in their optical quality. In the case of the apochromatic objectives this is not so. When these lenses were first designed by Professor Abbe, it was found convenient in the low powers to introduce a chromatic error which the ocular could correct. Consequently, if an apochromatic objective is used with an ordinary ocular it does not appear to be perfectly corrected, the actual condition being that the different coloured images are of varying dimensions. Most makers now arrange that their series of oculars, whether compensating or otherwise, are so made that when dropped into the body-tube of the microscope they are in their correct position—that is, no alteration of the mechanical tube-length is necessary, as shown in Fig. 20. It is for this reason that the compensating oculars of Zeiss are made in both the Huyghenian and Ramsden types; for it is only thus that the lower focal planes of the whole series of oculars can be brought into the same position when inserted in the draw-tube of the microscope. Also, when a change of eye-piece becomes necessary very little alteration of the fine-adjustment is required to bring each objective into exact focus.

The 'tube-length,' or the distance between the ocular and objective, in part determines the magnifying power of the system. Some English microscopes are fitted for what is known as the 'long' tube, with a distance of 10 inches or 250 mm. between the upper focal point of the objective and the lower focal point of the eye-piece. In Continental microscopes this tube-length is 160 or 170 mm. (usually the former), and this should not be varied unless for the purpose of correcting for variations in thickness of cover-glass. Further reference to this will be made when the methods of using the various optical parts are described.

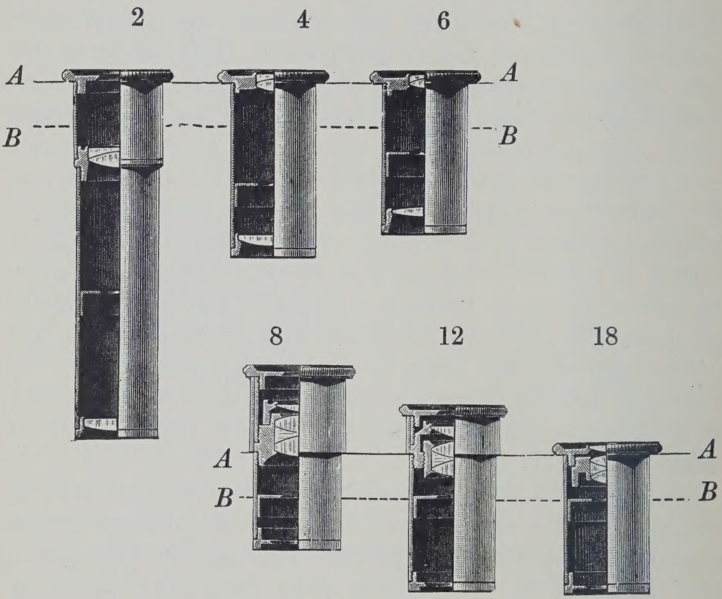


FIG. 20.—Compensating Eye-pieces. (C. Zeiss.) ½ full size.

A——*A*. Plane of the upper edge of the tube.
B.......*B*. Lower focal plane of the eye-pieces.

Compensating oculars are distinguished by numbers, which represent the number of times that the eye-piece magnifies the image formed by the objective when it is used with its standard tube-length.

Projection-oculars will be required for photography. When apochromatic objectives are used they are essential, but they work satisfactorily with any objectives made at the present time. In these the same principle is adopted as in the case of the compensating oculars, except that they are of low power, only amplifying the image from two to six times, and are constructed to project an image to a considerable distance. No ordinary ocular is designed to do this, although it will often do so without any loss of definition. Projection-oculars do not differ from the ordinary eye-piece in the way in which they fit into the tube of the microscope, and as already stated in reference to compensating oculars they are designated by a number which indicates their magnifying power. A projection-ocular, No. 2—which to obtain this amplification should be used on a short tube—will become approximately a No. 3 when used on the long tube, and no difference in the quality of the image is appreciable when used with either long or short-tube objectives. In each ocular is a diaphragm (Fig. 21), and by turning a milled head at the top of the ocular the image of this diaphragm can be focussed on the ground-glass focussing-screen of the camera. This operation must be carefully performed with each alteration of camera-length.

With any ordinary objective, which has an appreciable outstanding aberration of any description, an ocular tends to increase the imperfection of the resulting image. It is sometimes advisable in photographing to use oculars of low power, the necessary further amplification of the image being obtained by camera extension. Some achromatic objectives perform better without an ocular, and in such cases a longer camera is necessary, the required magnification being obtained by suitable camera extension.

With achromatic objectives of recent construction—e.g. with those which are sometimes described as semi-apochromatic—compensating oculars work well. In many of these lenses an approximation to the correction of the apochromatic lenses is obtained, and they are to some extent dependent on the oculars for obtaining the best results. Two firms have introduced a type of ocular which may be used with either achromatic or apochromatic objectives. These are called by Messrs. J. Swift & Son the 'Universal,' and by Messrs. W. Watson & Sons the 'Holoscopic,' eye-pieces. While they are of considerable use for visual work where a mixed series of objectives is in use, they cannot be recommended for photographic work. The alteration of correction is effected by altering the distance between the eye-lens and field-lens of the ocular; but at best they can only claim to be a compromise between the two recognised forms of ocular already described. A rough method of determining whether an ocular is of the compensating type, is to hold it up to the light—preferably to the sky: the image of the diaphragm then seen by direct observation will be fringed with a double band of red and orange; or, with the very low-power oculars, a yellowish edge only may be apparent. With ordinary oculars the same observation will usually disclose a well-defined blue fringe. Sometimes, to make this colour-fringe visible, the angle of incidence of the light in relation to the ocular may have to be varied, but usually it is apparent at once.

A point of considerable importance in visual work is the most suitable combination of objective and ocular. In photographic work this question is not so important, as the oculars are usually of lower power. It is an obviously simple method for obtaining increased amplification of the image to use a higher power ocular. Such a proceeding, however, if carried to excess, introduces many difficulties. In visual work the beam entering the eye with a high-power ocular is very much narrower than is the case with a low one, and consequently any small particles of dust lying within the ocular itself or the moving specks in the fluid of the eye become painfully visible. In photographing, these specks are most unsightly, as they usually have a broad diffraction-fringe round them. On the other hand, a discreet use of the method of increasing magnification by using a high-power eye-piece may be of great service. An example of this will perhaps best indicate the point.

If a one-sixth-inch achromatic objective, with an aperture of 0·75, is being used with a No. 8 ocular, this is probably the highest eye-piece that such a combination permits without appreciable loss of definition. If for this is substituted an

8-mm. apochromatic objective, which has approximately double the focal length of the other lens, and which has an aperture of at least 0·65, it may be used with an ocular of more than double the power without suffering in any way, and there would be little difference in resolution between the two arrangements. But there would be a very appreciable advantage when using the longer focus lens; more depth of focus would be obtained, and the correction of the image would be of a higher order. It must not be overlooked that nearly all one-sixth-inch ordinary objectives are actually of shorter focus than that—so that their aperture is relatively lower than it appears to be.

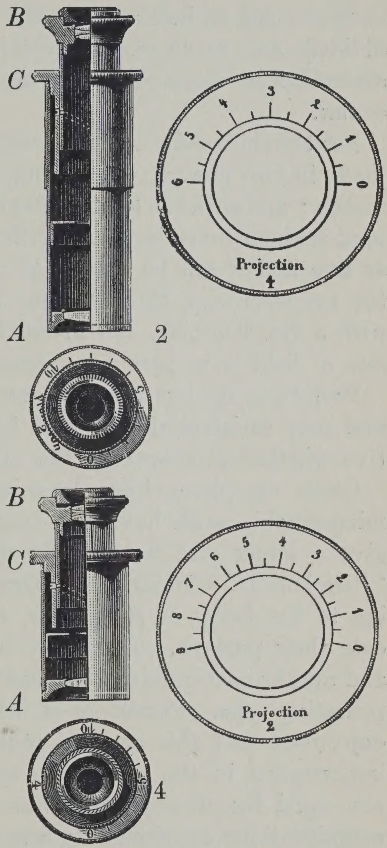


Fig. 21.—Projection Eye-pieces 2 and 4. (½ full size.)

This question of the best combination of objective and ocular must largely be determined by conditions, and would be influenced by the class of object that is being dealt with.

Fig. 21 shows the projection-oculars of Messrs. Zeiss, Nos. 2 and 4, in part section. The lens A is the collective-lens, and when the object is in focus produces, in combination with the objective, an image in the plane of the diaphragm in the ocular. The image of the diaphragm which is projected on to the ground-glass screen of the camera is focussed by rotating the top portion B of the ocular, and the amount this is moved can be read off on the scale which is in the position C. A reproduction of the scale is shown in the illustration in each case by the side of the respective oculars.

Messrs. Zeiss supply a tape-measure which may conveniently be utilised for determining, by measuring the camera length only, the exact position which the diaphragm should occupy in the ocular as indicated on the engraved scale. The scale is divided, and reads from 0 to 9; each division represents an alteration in distance of 0·5 mm. between the lenses of the ocular.

For the short-tube or Continental microscope these oculars are made in two powers, magnifying respectively two and four diameters; and with the No. 2 ocular the shortest camera that can be used is about seven inches. With No. 4 ocular a camera as short as five inches can be used. At a distance of one metre from the eye-piece cap, the field may be as much as 20 cm. across with a No. 2 ocular, and about 30 cm. with a No. 4, but this size of field is in part dependent on the objective used.

Projection oculars are now made by all microscope makers, and may satisfactorily be used in conjunction with any objective whether produced by the same firm or not.

Other eye-pieces have been introduced, which for photo-micrographic work have the considerable advantage that they give a larger and flatter field of view. They are known as 'Periplanatic,' 'Telluric,' or 'Orthochromatic' oculars, according to the fancy of the maker, names which sufficiently indicate their purpose. They may be used with both achromatic and apochromatic objectives, and so take the place of the compensating type. Curvature of field is an inherent defect in all objectives, and this type of ocular is designed to reduce the imperfection in the marginal part of the field. The benefit is not equal for all objectives, but is always appreciable. Their magnifications are similar to any other type of eye-piece, ranging from × 4, a very useful one for photo-micrography, to × 25. It is a more satisfactory method to describe oculars in terms of their focal length. The magnifying power may then be determined by dividing the focal length in millimetres into 250, the normal distance of distinct vision.

A series of oculars for projection and photo-micrography has been designed by Messrs. Carl Zeiss and is sold under the name of 'Homal.' The purpose of these also is to reduce curvature of field. Four ‘Homals’ have been produced, each of these working best with a particular objective. They cannot at once be applied to any microscope, in all cases some adaptation is needed. A flatter field is undoubtedly obtained, but where a large field is not particularly desired it is probable that the best results are to be secured with a projection-ocular. Messrs. Zeiss issue an interesting descriptive pamphlet to which reference may be made for full particulars of construction and use.

CHAPTER IV

THE OPTICAL EQUIPMENT:

CONDENSERS AND COLLECTING-LENSES

Sub-stage Condensers, and Illuminating Appliances.—It is mainly owing to the efforts of English microscopists that sub-stage condensers, for the efficient illumination of microscopic objects by transmitted light, have been brought to their present state of perfection. It was for long considered unnecessary to have any such arrangements as an essential part of the optical system, and even those that were used were of a crude description, their only function being to get a larger amount of light on to the object.

It is now recognised that the sub-stage condenser is second only in importance to the objective, and may be either non-achromatic, achromatic, or apochromatic. The focal length varies, as in the case of objectives, but this variation is confined to smaller limits.

In ordinary microscopes the non-achromatised condenser is still supplied for purposes of observation. While it fulfils its purpose well enough for visual work, it should, for photographic purposes, be discarded in favour of a more perfectly corrected combination. It is assumed that, whatever type is used, it is supported beneath the object-stage in the manner already described, and has both focussing and centring adjustments. If the additional expense of these adjustments in conjunction with an achromatic condenser is an objection, then better results would in general be obtained by using a reliable form of chromatic condenser with such adjustments, rather than an achromatic condenser without them.

The simplest sub-stage illuminator is the mirror which is fitted to all microscopes, and is on one side plane and on the other concave. By its aid, assuming that a large source of light is being used, parallel rays are projected on to the object when the flat mirror is used, and convergent rays when the concave side is in use. While no further arrangement may be necessary for the examination of simple objects under low powers, the mirror alone is quite inefficient for illuminating an object of any complexity or where other than the lowest powers are in use. For visual work it is an essential part of a microscope, but in photo-micrography it is thrown out of action when a horizontal type of camera is used. Even then it is essential for finding any particular part of an object, and for making preliminary adjustments when the microscope is separated from the photo-micrographic camera. For the lowest powers, using objectives of greater focal length than 1 inch, the concave mirror may be used, as unaided it will throw a sufficiently convergent beam of light on to the object. For any powers higher than this the plane surface of the mirror should be used. It is a safe general rule to use the concave mirror only when no other sub-stage appliance is necessary. It follows, therefore, that the plane mirror should always be used with a sub-stage condenser, so that it simply changes the direction of the light without altering the character of the illuminating beam. The simplest form of sub-stage condenser is that known as the Abbe illuminating apparatus, introduced so long ago as 1872. This is very easy to use, as it is not sensitive to moderate variation in its position in relation to the object so far as focussing or centration is concerned. It is, therefore, a convenient piece of apparatus for visual work, and may satisfactorily be used with medium powers for photo-micrography. With direct light it has many uses, but when used as an oblique illuminator—that is, with its central rays stopped out—it fails to fulfil its purpose in the way that better corrected condensers will do.

It is important in a condenser, as in an objective, that the numerical aperture should be large. The numerical aperture of the Abbe apparatus does not exceed 1·0; but its aplanatic cone does not exceed 0·45. It will be advisable here to explain briefly the difference between the total numerical aperture and the size of the aplanatic cone in sub-stage condensers, as it is rather on the latter that the efficiency of the apparatus depends.

Reference to Fig. 22 *a* will show that, with a simple uncorrected lens, the marginal rays do not come to a focus at the same point as the central rays: the lens, in fact, is not well corrected for spherical aberration. The diagram is of course exaggerated, as there is a portion of even a simple lens that will nearly bring the incident rays to one focal point; but it is obvious that this central portion is small in relation to the whole. The aplanatic cone, therefore, which is the largest cone of light that the lens will bring to one focal point with an illuminant of small area, is in this case a small one. Thus it may be assumed that the aplanatic portion of this condensing-lens is represented by the incident beam included between DD which focusses at A, whereas the outer portion between D and C will focus progressively nearer the lens according to its distance from the centre. The outermost zone C will therefore be focussed at B, the remainder somewhere between A and B.

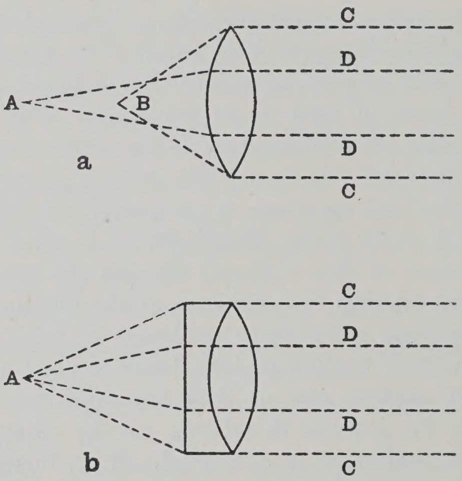


Fig. 22 *a* and *b*.—Diagram showing Correction for Spherical Aberration.

Fig. 22 *b* shows the lens system corrected for spherical aberration; the size of the aplanatic cone now approaches the total aperture of the condensing system, and the marginal rays are focussed either exactly or very nearly in the same position as the central rays. On the degree to which this is accomplished depends the size of the aplanatic cone.

As the primary object of the sub-stage condenser is to collect light from the illuminant and to project as much of it as possible on to the object, it is obvious that it is important for the aplanatic cone to bear as large a relation as possible to the total aperture. With condensers of the best type the aplanatic cone is little less than the total aperture, and in some cases is claimed to be equal to it. Where the cone is not aplanatic, considerable loss of light occurs; a certain amount of light becomes diffused and is not projected into the object-plane as it should be.

Illumination may be effected by two different methods: either by what is called a solid, or by a hollow, cone of light. A solid cone may be of widely varying dimensions; that is, it may have a narrow or broad base, the latter being as wide as the condenser itself is capable of transmitting. The cone is narrowed by closing the iris-diaphragm below the condenser, so that the light entering the condenser is reduced and confined to a greater or less proportion of its area. To obtain what is known as a hollow cone, the central rays are stopped out by introducing a central stop below the condenser varying in size according to the objective used. This results in the illumination of the object by an annular cone of rays, no direct light, but only that reflected or refracted by the object, entering the objective.

The deficiencies of the chromatic condenser are not only its want of spherical correction, but also, as its name implies, its want of colour correction. An achromatic condenser, therefore, gives an image of any radiant of a higher order than the simpler type. Achromatic condensers are to be obtained both dry and oil immersion. The dry ones have total apertures, usually of 1·0, and an aplanatic aperture varying from 0·9 to 0·95. They also vary in focal length; to obtain such a high aperture it has been found necessary to make them of short focus. The average focal length of a condenser of this type is one-fifth of an inch. The result is that the image of the radiant as seen in the microscope is small, and is not usually large enough fully to illuminate the field except with a high-power objective. To meet this difficulty most achromatic condensers are made so that the top combination may be removed, the effect being that the focal length is increased. The image of the radiant is therefore spread over a larger area, and consequently will illuminate an object evenly with a medium-power objective. This is a perfectly satisfactory method with most sub-stage condensers either dry or oil immersion, and their optical efficiency is not substantially lowered thereby. Their aperture is reduced and their focal length is increased, but their corrections are not sufficiently disturbed to interfere with their performance.

The removal of the top combination leads to a lowering of the numerical aperture; but, as this expedient is only necessary with lenses of medium power, their aperture being correspondingly lower, no loss results from the alteration. Among the best to be obtained is an oil-immersion condenser made by Messrs. J. Swift & Son. It has a total aperture of 1·40, and an aplanatic aperture of 1·30. Its focal length is $\frac{1}{4}$ inch. With the top hemispherical lens removed it may be used as a dry condenser of 0·62 N.A. Another worthy of note is one made by E. Leitz, of the same N.A., but the aplanatic cone nearly equals the total aperture. Both these condensers are capable of fulfilling the most exacting requirements. They are mentioned as examples; most makers now supply similar ones which are equally good.

Condensers which have been termed apochromatic have been brought out by one or two makers. The use of this name would not appear to be strictly justifiable, and probably arises from the fact that fluorite, and glass similar to that used in the construction of apochromatic lenses have been utilised. As these condensers do not equal the correction of the apochromatic lenses, for reasons that need not be entered into here, they are really not entitled to be so called. At the same time they are usually extremely good, and their performance is equal to that of a high-class achromatic condenser at its best, but there is no necessity for such a high order of correction.

It may be taken as an axiom that the illumination of an object should be effected with as large a solid cone of light as possible. No objective however—except when used for the observation of heavily stained preparations such as bacteria or similar objects with great contrasts—will stand illumination with a cone of light equal to its own numerical aperture. The effect of too large a cone is easily seen, as the object appears to be flooded in light and there is a general lack of contrast over the whole field. When this is the case the sub-stage iris-diaphragm should be contracted until fine details are clearly defined. If this process of narrowing down the cone of light is carried too far, diffraction-fringes will appear round the elements of structure of the object; and in no case should this point be reached (see Figs. 4 and 5, Plate XVII). The result of narrowing the cone of light by reducing the aperture of the condenser is in effect the same as that obtained by using an objective of smaller aperture. This is however not true for all objects, some highly refractile ones such as diatoms respond differently. If carried to excess, the size of the diffraction discs are so increased as to overlap; serious interference to the definition then results. This will be better appreciated if it is remembered that where broad beams of light are passing through an object, the amount of evident diffracted light in relation to the whole is not large, and it is not therefore sufficient to interfere with the general definition. Where a very narrow beam is passing through a narrow slit—which in effect is the condition that holds in objects with fine structure illuminated by narrow beams—the apparent diffraction becomes much larger in relation to the total amount of light actually passing.

It will be found in practice that the numerical aperture of the condenser should not exceed from two-thirds to three-quarters of that of the objective; usually a two-thirds cone will be sufficient. This does not mean that the whole aperture of the objective is not being utilised, for each detail of the object is producing its own diffraction image, and throwing broad cones or fans of light which are picked up and utilised by the objective. Consequently the effective working aperture is sometimes greater than the aperture of the sub-stage condenser itself, the extent of this depending on the optical character of the object.

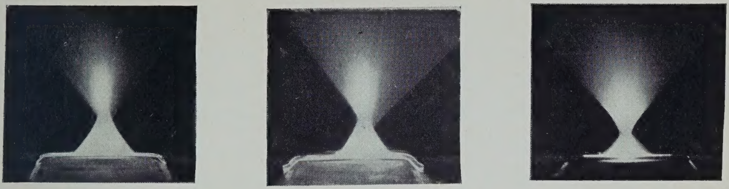
The proportion of the aperture of the objective which is being filled with light by the condenser may approximately be determined by removing the ocular from the microscope and looking down the body-tube. If the aperture of the condenser equals or exceeds that of the objective, then the back lens of the latter will appear to be filled with light; on the other hand, if the condenser aperture is less than that of the objective, there will be a greyish-black marginal zone, and in the centre of this a brightly illuminated area. The relation of the diameter of this bright area to the whole aperture indicates the proportion of the total available aperture being used.

Achromatic condensers are made either dry or oil-immersion in the same way and for the same purpose that objectives are so made. It is advisable to obtain a condenser of the highest possible aperture, since, as already pointed out, it can always be reduced in aperture by means of the iris-diaphragm and for low-power work the top lens can be removed. Thus a condenser combination will remain which is suitable for use with medium or low powers.

When adjusting the sub-stage condenser the image of the radiant may be projected and focussed in the plane of the object; but for many purposes, especially when photographing with radiants of small area, this method may be departed from. In focussing the image of the radiant, however, care should be taken that when it does not exactly coincide with the plane of the object, the apex of the illuminating cone falls above; the object, in fact, then occupies a position within the solid cone of light itself, and not above the apex of the cone.

The practical difference between these three types of condenser, an Abbe chromatic, an achromatic either dry or oil immersion, or an aplanatic, is what the worker really wants to know. With many objects the result is the same whichever is used; it is in fact impossible to see any difference in comparative photographs. The amount of light reaching the object is influenced considerably by the corrections of the condenser and this is exemplified in Plate I, in which the form of the cone of rays proceeding from each type is seen in section. A is the ordinary Abbe, and it will be seen that the fan-shaped portion above the point where the cone is smallest is uneven; this fan in fact indicates that there is an uneven distribution of light in the illuminating cone; it is more intense at the centre than at the periphery. It would follow therefore that an object occupying the point of intersection of the rays, as it should do, would receive more light from the centre of the cone than from its edge. This would not of necessity result in uneven illumination but it would mean that any element of structure in the object would not be illuminated in such a manner as to fully and completely utilise the aperture of the objective. With

PLATE I



A

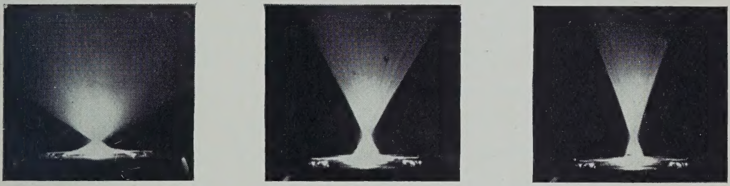
Abbe.

B

Abbe off Centre.

C

Zeiss Achromatic.



D

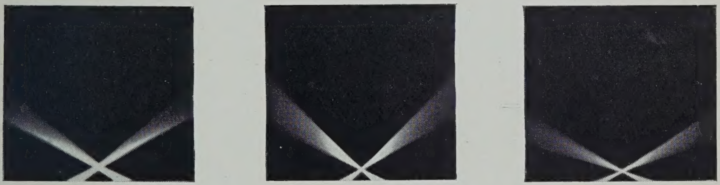
Zeiss Aplanatic Full Aperture.

E

Zeiss Aplanatic Iris 12 mm.

F

Zeiss Aplanatic Iris 8 mm.



G

Zeiss Paraboloid.

H

Leitz D.G.

K

Beck D.G. 1·27 N.A.

Cones of Rays formed by different Types of Condenser.

this uncorrected type of condenser, it is usually assumed that the light-source is large, such, for instance, as daylight affords, and this does to some extent reduce the disadvantage. In photo-micrography illuminants are usually of small area but high intensity, so that an uncorrected condenser is not satisfactory. Such objections become even more serious when stops of any shape are used, such as in the illumination of diatoms, as this implies that the outer zones of the condenser are operative. Thus it will be appreciated that an uncorrected condenser has considerable limitations, not the least of them being determined by the nature of the object itself. A further objection is that many microscopes fitted with Abbe condensers have no centring adjustment to the sub-stage. In many cases in fact they cannot be so fitted, the condenser itself being too large to admit of such adjustments. Fig. B shows the effect of imperfect centration, the errors in the condenser being accentuated thereby as the amount of light and angle of the beam reaching the object is greater on one side than the other. Such inaccurate illumination is usually due to incorrect centration of the illuminant, but in cases where it is not possible to centre the condenser correctly it may happen that no object can be properly delineated. The elementary principle of microscopic illumination, that each point in the object must receive from all sides and at all angles an equal quantity of light, cannot be complied with. Fig. C shows the improvement to be expected when a well-corrected achromatic condenser is used ; the corrections of the best of these being equal to those of an objective. Such are the only satisfactory types to use for visual work, but they have the relatively unimportant objection for photo-micrography, that they result in some loss of light as compared with the uncorrected type. This is due to the larger number of optical elements used in their construction, and consequent loss by absorption and reflection from each surface. Still such a condenser will meet every need, the beam is even throughout and can satisfactorily be used at its full aperture. Such a light cone will illuminate satisfactorily any point in an object and it can be stopped down by any method either at its centre or its periphery. Figs. D, E and F show the light-cone formed by an aplanatic condenser of N.A. 1·40, and this type is to be preferred both for micro-projection and for photo-micrography.

It will be seen that the distribution of light-energy in the fan-shaped beam is very even, and this determines its efficiency. Fig. D is the full cone, E and F being successively smaller cones as the sub-stage iris-diaphragm is contracted. These illustrations may make evident the real differences between these condensers, and the way in which the illumination of an object is effected. The matter is often dealt with in an abstract way, so the practical worker finds it difficult to follow.

The full N.A. of a condenser cannot exceed 1·0 unless it is in immersion contact with the under side of the object slide, the immersion fluid being cedar-wood oil as used with homogeneous immersion objectives. The object itself must also be mounted in a medium of sufficiently high refractive index, such as Canada balsam, or the full benefit of the high numerical aperture will not be realised. The intensity of illumination of the object, a factor of considerable importance and one on which exposure is largely dependent, is proportional to the square of the aperture.

Dark Ground Illumination.—For the illumination of unstained or living objects, some method of dark-ground illumination is desirable. Such methods depend on the utilisation of a hollow cone of light or by what is known as annular illumination. There are various appliances for this purpose, and some of these date back to the early days of microscopy. The simplest is that known as the ‘ spot lens,’ an arrangement suitable only for low-power work, and having no pretensions to be regarded as an accurate method of illumination. It consists of a condensing-lens of short focus, between which and the mirror of the microscope a black stop is placed at its exact centre ; the result is that any object occupying the position of the focal point of the lens is illuminated only by oblique rays, the central direct rays being obstructed. The proportion of the light-cone cut out by the central stop must be greater than the aperture of the objective used. Hence, on the assumption that the object is mounted in a medium having a refractive index substantially different from its own, any object placed at the apex of this illuminating hollow cone shows up bright on a dark ground.

The method of using a central stop in a suitable sub-stage condenser still remains one of the best for use with low- or medium-power objectives ; the advantage being that the stop may be varied in size to suit the objective in use (Fig. 23).

An appliance which allows the size of the central stop to be varied (much in the same way that the size of the opening of an iris-diaphragm can be varied) is known as the Traviss stop, and can be obtained commercially. A series of leaves are arranged at the centre, underneath the sub-stage, so that they can be opened or closed ; the size of the stop is thereby altered to meet varying conditions, or to suit any change of objective (Fig. 24).

This method of using the sub-stage condenser is only serviceable when its numerical aperture is substantially greater than that of the objective, as the illumination depends on the excess aperture of the condenser. Unless the excess is sufficient to transmit sufficient light to illuminate the object so that it is bright enough to be seen easily, the method is unsatisfactory. The stop used should be of such a size that it obscures more than the full aperture of the objective ; the illuminating area, therefore, consists of an annular zone which represents the difference in aperture between the objective and the condenser.

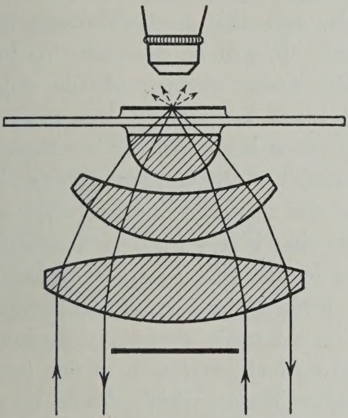


Fig. 23.

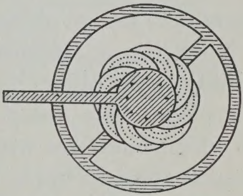


Fig. 24.

To obtain the maximum illumination and the best result, the size of the central stop must be suited exactly to the objective, and most makers supply a series of such stops for this purpose. In these the stop is supported beneath the condenser by a star disc, which is inserted in the stop carrier, and which has a central button pointing upwards. Each stop has a small central hole which drops over the button. It is important to see that the condenser is exactly centred, as on this depends the accuracy of the result. The iris-diaphragm should be fully open except when using low-power objectives, with which a better result is often obtained by partially closing it. This is however only a matter of ordinary observation; the appliance can be regulated to obtain the best effect. An oil-immersion condenser with an N.A. of at least 1·30 is suitable for this purpose and with this arrangement objectives as high as a one-sixth inch may be used. The light must be so projected that the back lens of the condenser is completely filled with light. It is obviously on the use of the marginal portions of the condenser that the illumination depends, and this part therefore must be completely and evenly filled. It will be necessary to bring the condenser close up to the under surface of the object-slide, the thickness of which must be slightly less than the focal length of the condenser. It is important to see that the central stop is at the optical centre of the condenser, since any want of centration is fatal to the resulting image. It is also important to use an object-slide that is free from dirt, scratches, and impurities or irregularities in the glass; any such become as visible as the object itself. Even if they are not in the exact object-plane, they result in considerable general diffusion of light which interferes with the optical perfection of the image. The same remarks apply to cover-glasses, which should be perfectly free from defects, and their thickness must be that for which the objective is corrected. In setting up the condenser and putting the cedar-wood oil between it and the object-slide no air-bubbles must be included.

The object is usually mounted in water or in a fluid with a similar refractive index. The preparation should be as thin as possible and the mounting medium free from air-bubbles or turbidity.

It is usual with annular illumination to employ an objective as low in power as possible, the necessary further amplification of the image being effected by the use of higher power oculars.

For photographic purposes a high-power source of light is essential; and, where the exposures are to be short, the only practicable light is the electric arc. The correct centration of the light in relation to the optical system must be carried out with the same care as when photographing any other microscopic object.

It may sometimes happen that the amount of light transmitted by the marginal portion of the condenser is greater than the object requires. This may be counteracted by closing the sub-stage iris-diaphragm until the illumination is satisfactory.

The best modern dark-ground illuminators for use with high-power lenses, and which are suitable for the illumination of living bacteria or similar unstained objects, are of the concentric reflecting type now made by nearly all manufacturing opticians.

One exception to these (shown diagrammatically in Fig. 25 *a*) is an oil-immersion paraboloid, practically the same in design as that which has been in use for many years. The apparatus is calculated to reduce both spherical and chromatic aberration. The illumination is effected by internal reflection of the light within the paraboloid, and relies only to a very small extent on refraction. Reference to the figure will indicate clearly the path of the rays, and it will be seen that the object lies at a point where the whole of the light is concentrated. This apparatus is simple to use providing that certain essential points are attended to. Centration must be carefully carried out, and Messrs Zeiss recommend that the objective should be supported on a centring nose-piece and that centration should be by this means rather than by the ordinary sub-stage centring arrangement of the condenser. If, however, the sub-stage itself is carefully centred, it would not appear that the objective requires any such adjustment; but in some micro-

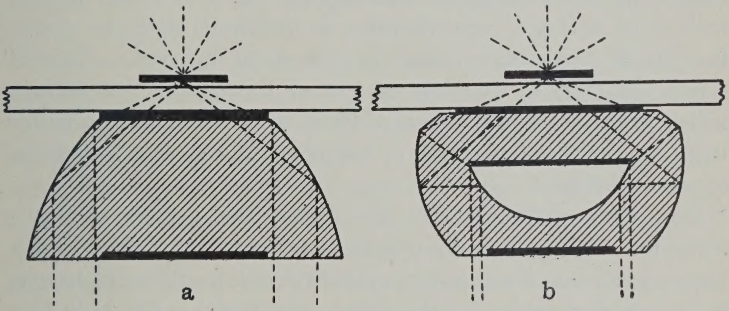


FIG. 25.—Diagram of Path of Rays (*a*) in a Paraboloidal Condenser; (*b*) in Dark-ground Illuminator with Spherical Surfaces.

scopes the centring sub-stage will not carry the paraboloid. The thickness of the object-slide must be that for which the condenser is designed, and must not vary more than 0·1 mm. As in all dark-ground methods, when using high-power dry lenses, exact correction for thickness of cover-glass must be secured either by collar adjustment in the objective itself, or by alteration of optical tube-length. The same precautions must be taken to eliminate any defects in the object-slide or cover-glass as in other dark-ground methods.

A condensing-lens must be used between the source of light and the paraboloidal condenser, so that its posterior surface is completely and evenly filled with light. A one-sixth-inch or 4-mm. apochromatic objective may be used with this illuminator ; but if an oil-immersion objective—such as a one-twelfth-inch or 3- or 4-mm. apochromat—is preferred, then the objective itself must be reduced in numerical aperture. This is simply effected by putting in one of the small cone fittings which drop inside the objective itself, and which have already been described as a method of reducing the numerical aperture of an objective.

More convenient methods of reducing the aperture of objectives for dark-ground illumination are now available. Both Leitz and Zeiss make objective mounts in which an iris-diaphragm is embodied, operating at the correct position near the back lens of the objective. A separate mount can be supplied to which the optical part of the objective can be attached, or the objective with iris mounted in it is made as a complete unit. Such an objective can be used either for bright- or dark-ground illumination, but for the former it is important to see that the iris is open to its fullest extent or the full aperture of the objective will not be available. It is useless to attach an iris-diaphragm at any point other than near to the back lens of the objective. The drop-in stops are the only alternative.

One type of concentric condenser is shown diagrammatically in Fig. 25 *b*, where the path of the rays is indicated. It will be observed that the method depends on total reflection within the apparatus itself, the light being ultimately condensed on to the object. It is sensitive to variation of thickness of object-slide, and in use all the points already enumerated should be attended to. The results to be obtained with it are satisfactory in every respect, and, owing to its construction, it is possible to secure a more exact concentration of the rays and a higher degree of correction than with the paraboloidal form.

In the case of diatoms, or similar objects, it is often necessary for the illumination to be in one azimuth, and for this purpose stops are designed which allow of the utilisation of certain portions of the cone of light passing into the objective, the remainder being stopped out. As this method is of rather special application, further reference to it will be made when dealing with the methods of photographing particular objects.

It must be understood that dark-ground methods do not result in any increase of resolution beyond that of the objective when stopped down, although they may render visible isolated particles or the components of irregular structure that are not seen easily by other methods.

The path of rays in dark-ground illuminators, photographed in section in the same manner as the ordinary condensers already described, is shown in Plate I, Figs. G, H and K. The paraboloidal type is seen in G, and it is evident that while the concentration is good and the amount of light collected is large, the order of correction is not equal to the following two, H and K. As the point of intersection of the rays is both broad and deep, it follows that the adjustment need not be so accurate as with the spherical surface concentric condensers. This accounts for the fact that paraboloids are often preferred by laboratory workers as adjustment is easier and thickness of slide can be varied within wider limits. If the beam is carefully observed, however, as it proceeds upwards in the figure, it will be evident that the light distribution is not even, it tends to fall off from one edge to the other. This is an indication that the light is not impinging on to the object equally at all angles; such a condenser is therefore not desirable for the most accurate work. The concentric type, Fig. H, is more perfectly corrected, in fact, if properly made and centred it is as free of aberrations as any optical system can be in practice. This is due to the use of reflecting surfaces only, the difficulty which exists in the perfect correction of refracting elements not being met with. Fig. K shows the beam from the recently introduced illuminator made by R. & J. Beck, Ltd. This is made to work with an objective of 1·20 N.A. and is the most perfect of this type hitherto produced. It is not suitable for ordinary purposes, since to get the full beneficial effect of the ability to use an objective of higher N.A. much greater care must be exercised in setting it up on the microscope. Thin slides are necessary, certainly not more than 0·5 mm., and this limits its use to preparations on slides of this thickness. For those, however, who wish to get the best results, regardless of any little extra trouble involved, this appliance is of great value. In photographing the exposure is of necessity lengthened, owing to the narrowness and obliquity of the beam, and consequent smaller amount of light reaching the object. This condenser is provided with an adjustment for various slide thicknesses, but the high N.A. is only to be obtained if a thin slide is used as already mentioned.

A type of dark-ground illuminator is made by Messrs. W. Watson & Sons to the design of Mr. E. M. Nelson (Fig. 26). It is known as the 'Cassegrain' illuminator because its curved reflecting surfaces are disposed somewhat similarly to the mirrors of the reflecting telescope invented by Cassegrain.

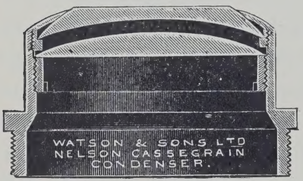


Fig. 26.

The main advantage claimed for it is that it enables an illuminating beam of great obliquity to impinge on the object so that objectives of the highest N.A. can, under suitable conditions, be used. Further, its construction is such that a large light source, such as a frosted electric lamp, can be used with it and the quantity of light transmitted is sufficient for most purposes. Apparently its correction is not so perfect as some other reflecting condensers so that precise concentration of the light on the object at the point of observation is not obtained. It is undoubtedly a condenser of value for some purposes, as it is not always necessary to have the high correction that a well-made concentric reflecting condenser gives. It is said to work satisfactorily with an objective having an N.A. as high as 1·37, the object being mounted in a medium such as Canada balsam. With objects mounted in water or in fluids of similar refractive index the advantage of this condenser is not so obvious.

The resolving limit in the microscope may be found by multiplying the N.A. of a given objective by 40,000 when monochromatic green light is used, and illumination is by a solid axial cone of light. With oblique illumination in one azimuth and with a small portion only of the peripheral zone in use, the power of resolving regular structure is increased by about one-third; but this latter method is practically only applicable to the illumination of objects such as diatoms in which there are considerable diffraction effects in the objects themselves. The limit of visibility, so far as isolated objects are concerned, is quite a different point, and Lord Rayleigh states that isolated objects, or two dark areas separated by a bright line, may be seen if the line is as narrow as one-sixteenth and under certain conditions one thirty-second of the wave-length of the light used, although the resulting image does not of necessity represent the actual appearance of the object. The chief factor under such circumstances is the intensity of the illuminant, and there is much reason to conclude that any isolated object may be rendered visible, even if it approaches molecular dimensions, provided that it is able to reflect sufficient light for the eye to appreciate. A keen observer will see all the structure in a microscopic image that the best objectives can show with a magnification of 750 to 800 diameters, although it may facilitate observation to amplify the image beyond this.

Opaque and Vertical Illuminators.—The methods of illumination so far described are applicable only to transparent objects; for opaque objects totally different appliances must be used. Opaque illuminators are placed above the microscope stage; in the case of low powers between the object and the objective, and for high powers as near as possible to the posterior combination of the objective. The specimen may be illuminated either by obliquely reflected or normally reflected light.

In general the principle of illumination of opaque objects is to project on to the surface of the object a reduced image of the illuminant; as nearly as possible securing the same conditions as in the illumination of transparent objects, except that the image formed is not the result of light obstruction, but is due to the differences in absorption or reflection by the structure under observation. The appearance obtained, apart from the structure of the object itself, will vary according to the angle of incidence of the light. If low powers are used, the simplest method is to project on to the object, by means of the ordinary bull's-eye, a beam of light of greater or less obliquity to the optical axis. Assuming that the surface of the object has some irregularities of structure and is not a highly polished metallic surface, the image will consist of alternate areas of light and shade. If, however, the beam is projected vertically and in alignment with the optical axis of the microscope, the appearance will be altered and in general a reversal of the light and shade will take place. The result of the alternative methods of illumination—at least, with metallic surfaces —is practically positive and negative. It was almost entirely due to the demand in micro-metallurgy for improved means of illuminating opaque objects that the existing methods and appliances were originated, and therefore the illustrations are mainly those of metal surfaces.

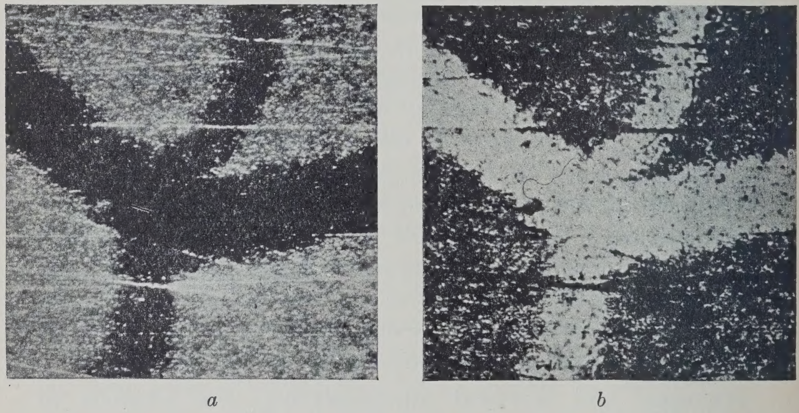

a *b*

Fig. 27.—Frosted Silver-leaf Film on Glass; (*a*) by Oblique Light, (*b*) by Normal Light. × 250.

Fig. 27 *a* and *b*, which are photographs of a metallic surface illuminated by oblique and normally reflected light, will illustrate this point, and it will be seen that the appearances are practically reversed so far as light and shade are concerned.

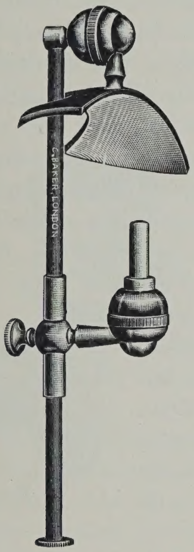
The reason is fairly obvious; when the illumination takes place by projecting the light at an angle of approximately 45° to the surface the beam is reflected and no direct light enters the objective unless some alteration of its direction is caused by the structure or irregularities of the object itself. A smooth polished surface will under these circumstances appear practically black. If now the same object is illuminated by a vertical illuminator, the conditions are reversed; the rays are condensed on to the surface of the object by means of the front lens of the objective, and are totally reflected by the polished surface, passing up through the objective again and causing the surface of the object to appear brilliantly illuminated. The specimen selected to illustrate this point consists of a frosted silver surface on which a burnisher mark has been made. In both these images a certain amount of light is scattered; but where the burnisher has passed over, the irregularities are almost obliterated, with the result that this mark under oblique light appears black, and with vertical illumination appears as a totally reflecting surface. Even a metallic surface is, however, not perfectly opaque, so that structure becomes visible as the result in part of refraction or absorption. The absorption may be general, giving an effect of light and shade; or it may be selective, with the result that if light of a particular wave-length is absorbed, either over the whole surface or over small portions of it, the image is a coloured one. A complete comprehension of the variations in appearance to be obtained by these methods of above-stage illumination is most essential in order to interpret the microscopic appearances of opaque objects.

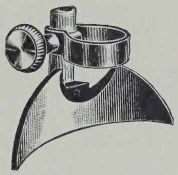
Where an object is a perfectly plain and highly polished etched metallic surface, both methods—the oblique and the vertical—should be used for its examination. With the oblique method the angle of incidence is determined by the focal length or working distance of the objective; in fact, the applicability of this method depends entirely on the distance between the front lens of the objective and the object. If this distance is very small, the incident ray becomes so nearly horizontal that little or no reflection of the light into the objective takes place. For high powers, therefore, the vertical method or some modification of it has to be resorted to. When using objectives of low power—certainly not higher than half an inch—the method of projecting the image of the radiant directly on to the object by the bull's-eye condenser is practicable. For opaque objects, such as foraminifera, where well-defined shadows can be obtained, this method works fairly well; but it is always open to the objection that on one side a hard shadow is obtained from which little or no light is reflected into the objective. Where no other arrangement than the bull's-eye is applicable, this can be obviated in part by ensuring that the light is as high above the level of the stage of the microscope as the objective will permit, so that the shadows are reduced to a minimum. The bull's-eye should be in such a position that the smallest possible image of the radiant that will illuminate the field is projected on to the object. Heavy shadows can be reduced by placing a small piece of white paper near to the object on the side opposite to the bull's-eye. Thus some diffused light is thrown back on to the unlighted side.

A simple arrangement that gives good results with opaque objects, and is an improvement on the bull's-eye method alone, is the parabolic silver side-reflector. It consists of a concave piece of metal silvered on its inner side, and is so placed above the object that a beam projected by the bull's-eye condenser is condensed by the internal reflecting surface, and the light is thus thrown on to the object (Fig. 28). It may be attached to the microscope body or placed on the stage, and is usually supplied with a ball-and-socket joint. The advantage is that the illumination, although still confined to low powers, is not at such an acute angle to the surface of the object as when the bull's-eye alone is used.

A further development of this method is known as the Sorby parabolic reflector (Fig. 29). It consists of a parabolic surface with an auxiliary reflector, the latter arranged so that it can be turned out of action if desired. This reflector is suitable for powers not higher than one inch, and is made to slide on to the mount of the objective. It is an improvement, and gives better illumination than the simple side-reflector already described, and is particularly applicable to metallurgical work. With both these side-reflectors a bull's-eye must be used to throw a parallel beam on to the reflecting surface, and it is essential that the projected parallel beam illuminates the whole of the reflector. The position of the radiant should be such that the smallest possible image is thrown on to the surface to be illuminated. The best results are obtained when the reflector is specially made for the particular objective in use

—a very brilliant image then resulting. It throws light upon the object over nearly 180° of arc, and thus illuminates a considerable amount of a solid object at one time, and lessens the shadows that are obtained by the method in which the bull's-eye condenser alone is used. If it is made for and mounted on the objective, it is simple in use as no adjustment is required; but where only one reflector is used for lenses of varying focal lengths, it slides up and down on the objective until correct adjustment is obtained.





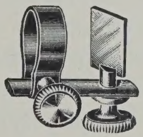


Fig. 28.

Fig. 29.

With the Sorby parabolic reflector the flat silvered mirror, which swings in and out as described, results in the light being thrown down directly on to the object, and thus a close approximation to vertical illumination is obtained. The mirror obscures one-half of the front lens of the objective, but by throwing out the auxiliary reflector it admits of a rapid change of illumination from oblique to vertical light; it is therefore very useful for ordinary purposes of observation.

Another arrangement for top-lighting—which, however, is only applicable to small objects—is known as the Lieberkuhn illuminator (Figs. 30 and 31). This is a concave silver reflector which slides on to the mount of the objective; this again must be made for the objective in use, as its curve must suit the focal length of the latter, or more exactly its working distance. The illumination is effected from below the stage, the light being projected upwards by the flat surface of the mirror on the microscope as for transparent objects. The object must be so small that it allows sufficient light to pass round it for the Lieberkuhn to be completely filled. The light on striking the silvered surface is reflected from all sides and condensed on to the object, and the best results are obtained when a dark-stop is placed below the object, intercepting the central portion of the incident beam. The performance of this apparatus is at its best when the conditions indicated are fulfilled; it then forms a very useful method of illuminating opaque objects where such illumination is required to be incident from all sides. It is only suitable for use with low or medium powers. Considerable modifications and improvements of this type of illuminator have been introduced and descriptions will be found in Chapter XI.

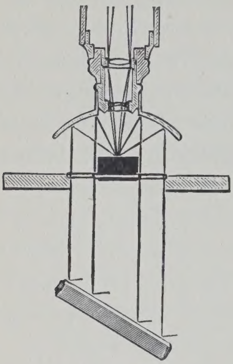


Fig. 30.

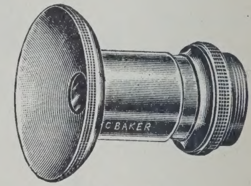


Fig. 31.

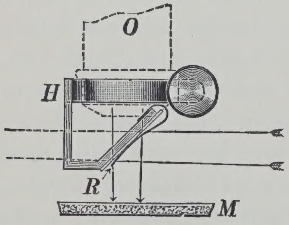


Fig. 32.—Reflecting Illuminator for Low Powers.

The simplest method of obtaining normal illumination with low powers is by placing between the object and the objective a cover-glass, or piece of thin optically worked glass, at an angle of 45° to the optical axis (Fig. 32). The light is thrown in the direction as shown, striking the reflecting glass surface R, and is partially thrown down on to the object M. It is reflected from M in the direction of the optical axis, and is received by the objective O. The appliance is clamped on to the front of the objective by the metal ring H, and is perfectly efficient for any power where there is sufficient working distance for it to be placed in position. The illuminant with which it is used must be of fairly high power as there is considerable loss of light.

For the illumination of opaque objects, for observation with high powers, recourse must be had to one of the two available types of vertical illuminators (Fig. 33 *a* and *b*). When using these the object should either be in optical contact with the cover-glass or should be uncovered—preferably the latter. Recent improvements in the illumination of opaque objects and in the methods of obtaining variations of the beam of light incident on such surfaces, have been due to the examination of metal surfaces by such means.

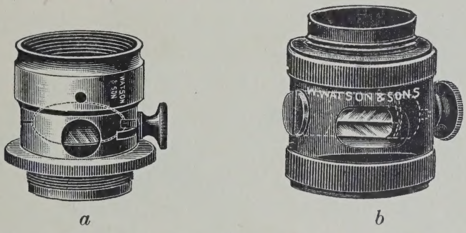


Fig. 33.—Vertical Illuminator for Opaque Objects. (W. Watson & Sons.)

The vertical illuminator is attached to the microscope in the position usually occupied by the objective, and the objective is then screwed into its lower end. It therefore occupies an intermediate position between objective and ocular, but is situated as near as possible to the former. It consists essentially of either a thin cover-glass, an optically worked piece of plane glass, or a small totally reflecting prism. A beam of light is projected at right angles to the axis of the microscope so that it falls on to the reflecting surface and is thrown down through the objective on to the object, on which an image of the illuminant is formed. The objective thus performs a double function ; it acts as a condenser, while also serving its ordinary purpose of image-formation and magnification of the image. The surface of the specimen may then be viewed through the eye-piece of the microscope in the ordinary way. Whether the cover-glass or the prism is the better form to use is a controversial question ; but the prism arrangement certainly has the objection that one-half only of the objective is in use. The prism is so arranged that it projects over one-half of the back lens of the objective, and this half therefore serves as the condenser; the remaining half forms the microscopic image. In general, it will be found that the arrangement in which a cover-glass is used has some advantage, as there is no interference with the aperture of the objective. The carrier containing the reflecting arrangement is made so that it may be rotated, and the angle of incidence of the light may be varied within limits. The radiant should be approximately eight to ten inches from the microscope, since it is only at a distance such as this that the objective will efficiently condense the light on to the object. To obtain exactly vertical illumination the illuminator should be in such a position that its reflecting-surface is at an angle of 45° to the vertical.

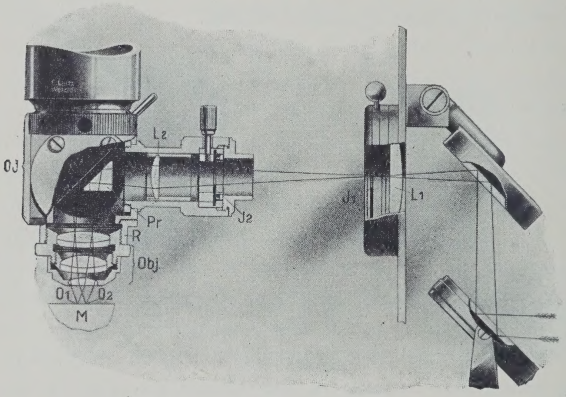


Fig. 34.—Vertical Illuminator for Opaque Objects. (E. Leitz.)

An improved vertical illuminator has been brought out by Messrs. E. Leitz (Fig. 34). It consists essentially of the same arrangement as that already described. It has, however, inserted at one side, and in the direction in which the light is thrown on to the prism, a subsidiary tube carrying a small condensing-lens L2, which is adjustable in position in the direction of the length of the tube. To be used in conjunction with this appliance is a separate arrangement consisting of two mirrors; the lower one is adjustable in any direction, the upper one is arranged at an angle of 45° to the vertical. The incident light, therefore, on striking the first mirror is reflected on to the second and thrown into the condensing lens L2 of the vertical illuminator. The advantage is that the position of the source of light in relation to the whole apparatus is not of primary importance. The aperture J1 has to be centred so that a parallel beam is thrown on to the lens L2, but after this has been done the light may be thrown on to the lower mirror and is variable in direction within considerable limits.

An additional condensing-lens L1 is also arranged, so that it may be thrown into action over the aperture J1; it enables the light to be condensed and a higher degree of illumination to be obtained. At J1 an iris-diaphragm is placed so that the width of the incident beam may be varied. The apparatus can be used with either the prism or the cover-glass type of illuminator, both of which are supplied.

Vertical illuminators require very considerable care when used for photo-micrography, as there is great danger of a considerable diffusion of light occurring at the object or at one of the many reflecting surfaces in the optical system. For this reason the best results are obtained by using these illuminators in conjunction with oil-immersion objectives; the number of reflecting surfaces is thereby reduced, and there is no chance of light being diffused between the front of the objective and the object, for the object is in this case directly immersed in the cedarwood oil. While this diffusion is sometimes not of importance for visual work it is extremely troublesome when a photograph is required, and it is essential that the exact conditions should be fulfilled under which the appliance works at its best.

A modification of the two forms described has been suggested by the late Dr. Rosenhain. The general construction is similar to the vertical illuminators described, but his reflectors consist of differently shaped thin silvered surfaces, either circular or annular, and they are mounted in such a manner that they may be shifted either laterally or vertically. Great importance is attached by him to the necessity of having the reflecting surface as close as possible to the posterior combination of the objective, and where particular appearances are required the adjustments by which the reflectors may be swung to one side or the other are useful.

A further development of this method has been introduced by Messrs. R. & J. Beck, Ltd. This provides for interchangeable reflectors of various shapes and sizes. Some are flat, others concave, and they can be had transparent, semi-silvered, or opaque, to suit any requirements. These reflectors are mounted to secure accurate adjustment in all directions, and can be used on any microscope. The illumination of metal surfaces is in large part a question of trial and error. The wide range of reflecting or absorbing surfaces, differing in colour and in contour, makes this work difficult if good results are to be secured. It is therefore advisable to experiment with all available appliances until the desired result is obtained. The same principles apply as to all microscopic work, the results depending entirely on accurate technique.

In photo-micrography too much attention cannot be given to illumination, for on this more than on any other factor depends the perfection of the result. No apology is therefore offered for having given a rather lengthy and detailed description of practically all illuminating appliances available at the present time. In succeeding portions of this book where the photographing of special objects is described, the method of illumination will in all cases be as fully set out as is practicable.

The Collecting-lens System.—The satisfactory illumination of microscopic objects for photographic purposes is not without certain difficulties which are due to the size and shape of the illuminant. The field of view must be evenly illuminated, and as a rule the illumination must be more intense than in visual work. Most illuminants of high power are small in area; consequently they do not sufficiently cover the field unless an enlarged image of the light source is used. For this reason some auxiliary collecting-lenses are necessary, and a description follows of the principal types.

The simplest form of collecting-lens to place between the source of light and the sub-stage condenser of the microscope is the bull's-eye. This is a plano-convex lens of short focus which is placed so that it projects a practically parallel beam of light into the posterior combination of the condenser. The plane side of the lens should be turned towards the light. It is usually supplied with vertical and horizontal adjustment so that it can be placed in correct alignment with the other portions of the optical system. It is useful for low powers, and for work not exceeding one hundred diameters will be found of service, but, owing to the fact that it is uncorrected it is not suited for use with high powers.

An improved type of this appliance designed by Mr. E. M. Nelson may with advantage be substituted for the ordinary form. In this the condensing system is composed of two lenses so that spherical aberration, inherent in the single bull's-eye, is considerably reduced, and the illumination obtained is of increased brilliancy. It is usually supplied with centring-screws similar to those used for sub-stage condensers, as it is much more sensitive to errors of centration than the ordinary type. The plane or concave side should be towards the source of light, as already indicated for the ordinary bull's-eye. It has proved to be a useful accessory, and will fulfil the requirements of a photo-micrographer where the more elaborate condensing systems to be described are not available.

The Watson-Conrady collecting-lens system is one that has some interesting characteristics. In this the lenses are purposely kept small, on the ground that with large lenses, especially those uncorrected for spherical and chromatic aberration, a great amount of the light passed cannot be utilised in the microscope. The system consists of a corrected achromatic lens of $2\frac{1}{4}$ inches diameter and $3\frac{1}{4}$-inch focus, by which a sharp enlarged image of the source of light can be projected in the position of the iris-diaphragm in the sub-stage condenser. As this lens comes so close to the source of light and is apt to get very hot, it cannot be cemented with Canada balsam in the usual way, but has to be put together with glycerine. The glycerine will resist the heat for a considerable time, and in the event of evaporation can be replaced by the user.

If reference is made to the figure illustrating the parts of this apparatus (Fig. 35), it will be seen that the image of the radiant produced by the condenser A is focussed in the plane of an iris-diaphragm C, by means of which the beam can be reduced to such a diameter as just suffices to cover the object which is being photographed, and consequently any unnecessary light is kept out of the field of the microscope. Another iris-diaphragm B is placed close to the condenser itself, and by closing this the width of the beam of light can be adjusted so that the sub-stage iris of the microscope is filled with light. By the combined use of these two iris-diaphragms the light reaching the microscope is limited to that actually in use when taking the photograph. An auxiliary single lens is placed close to the iris-diaphragm on which the image of the radiant is received, and this lens is of such focus that it produces on the iris of the sub-stage condenser D of the microscope a fairly sharp image of the large aplanatic condenser. This apparatus has been found to fulfil all that is claimed for it, and where it is convenient to have the radiant at some distance from the microscope it is very useful.

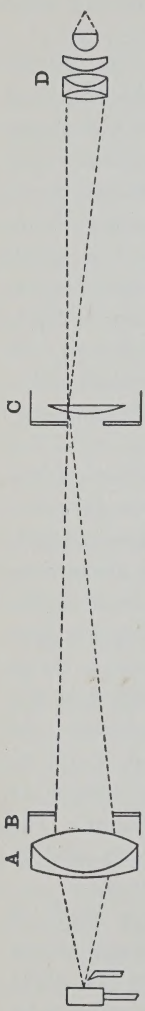


Fig. 35.—Path of rays in Messrs. Watson's Subsidiary Achromatic Collecting-lens.

A complete collecting-lens system that may be used with objectives of any power is made by Messrs. Zeiss to the computation of Dr. Kohler. Three lenses are used, and are distinguished by the Roman figures I, II and III. By arranging these lenses in different ways they can be used with any illuminant, whether of large or small area. For work with high powers, lens I is used singly as a simple collector. It is arranged to project the image of the radiant (assuming the latter to be an arc light or some similar illuminant of small area and high power) upon the diaphragm of the sub-stage condenser of the microscope, magnifying it about four times. In adjusting this it should be so placed that the image of the mount surrounding the collecting-lens is projected by the sub-stage condenser of the microscope into the centre of the field of view. Fig. 36 *a* shows the arrangement of lenses, etc., set up for high-power work. In this arrangement the image of the radiant should be so large that the back lens of the condenser is filled with light, as otherwise its full aperture would not be utilised. But, on the other hand, when using short-focus sub-stage condensers, the image of the radiant projected in the field of view is small, and is therefore only sufficient for illumination when using high powers.

For moderate magnifications lenses I and II are used in combination (Fig. 36 *b*). Lens I is brought so close to the source of light that the latter is approximately at the focal point of the lens and projects a parallel beam in the direction of the microscope. Lens II is now placed in such a position as to receive this parallel beam, collecting the light and projecting an image of the radiant on to the diaphragm of the sub-stage condenser of the microscope. In this case the projected image is smaller than where lens I only is used, and consequently the aperture of the sub-stage condenser is somewhat reduced, but as the arrangement is for medium powers only, this is of no moment.

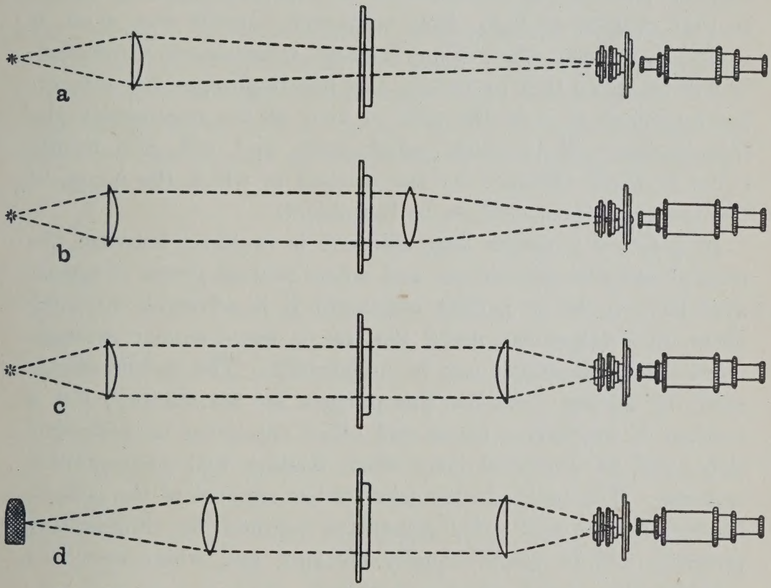


Fig. 36 *a, b, c, d*.—Collecting-lens Systems of Great Light-gathering Power.

For low magnifications with lenses from $\frac{1}{2}$ to 1 inch, where a relatively large area has to be illuminated, lenses I and III are used in conjunction (Fig. 36 *c*). These lenses are of such focal length that the image of the source of light projected on to the iris-diaphragm of the microscope is the same size as the radiant; consequently it is only useful for the powers indicated, owing to the sub-stage condenser being but partially utilised. It is important to have an iris-diaphragm to cut off extraneous or stray light, and it should always be placed approximately in the position shown in Fig. 36. Such a diaphragm is advisable in any case, as it can be regulated to allow the field of view of the objective to be just filled and no more; so that extraneous light, from whatever cause it may arise, is excluded, and the effect of any internal reflections is eliminated. It will be found that by closing this iris-diaphragm and projecting an image of it in the field of view of the microscope, the illumination will be quite satisfactory, and will give results equal to those obtained by the method in which the image of the radiant is projected on to the object.

In practice a rather long distance is required between the radiant and the microscope, and where several pieces of apparatus have to be in perfect alignment it is advisable to carry them on a triangular metal bar, or on some similar arrangement where alignment can be maintained. The saddle stands made by Messrs. Zeiss for this purpose are satisfactory, but a method of supporting lenses and other apparatus on geometric slides will be described later when dealing with photographic cameras. This method when used for the support of the collecting-lens system and other apparatus required for illuminating purposes will be found equally efficient, and where cost is a consideration, much more economical.

A diagram is also appended (Fig. 36 *d*) showing the arrangement which is necessary when incandescent gas is used as the radiant, or any similar source of light of large area and low intensity. The illustration is sufficiently descriptive and requires no further explanation.

Where the cost of the Kohler system of lenses is an objection, an efficient substitute may be arranged by fitting up three ordinary non-achromatised lenses. These may be purchased for two or three shillings each from most opticians. Two plano-convex lenses of about 10 to 12 inches focal length, and one bi-convex of about 18 inches focus will be found suitable. They may in use be set up in the manner shown (Fig. 36), and will be found more efficient than an ordinary bull's-eye condenser. This will again be referred to when dealing with working methods.

An aplanatic collecting-lens is made by Carl Zeiss which is an improvement on anything hitherto produced. It is aspherical, that is, its correction depends on the curvature of the lens surfaces and not on a combination of lenses. It will be found of considerable value in photo-micrography or for micro-projection, as it has great light-transmitting power. With this lens an arc-lamp taking five amperes is sufficient for almost any purpose. When radiants of large size are used, or where a diffusing surface such as ground glass is regarded as the light source, simple condensing-lenses are quite satisfactory. With small light-sources, such as an arc-light or Pointolite lamp, the aspherical lens or some similar aplanatic condensing-lens should be used.

CHAPTER V

SOURCES OF ILLUMINATION

The illuminant is perhaps of primary importance, and will to a great extent determine the class of work attempted; difficulties incidental to the use of high powers are considerably increased unless a light source of sufficient intensity is used.

There are many sources of illumination available, and these will be dealt with somewhat in the order of their intensity.

In selecting the illuminant it should be borne in mind that photographic plates can now be obtained of much greater sensitiveness to any portion of the spectrum than was formerly possible. At the same time (as will be pointed out in considering the selection of suitable photographic plates) a slow plate is desirable, so that, broadly speaking, the more powerful the source of light, the better the photographic result. It is also stated by no less an authority than Professor Abbe that the more powerful the source of light, the better the resulting optical image. On the other hand, for low-power work, and particularly in cases where the objects are thin or transparent and lacking in contrast, a less powerful light may give the best result.

In visual work it is now recognised that some method of altering the intensity of the illuminant without altering its quality is desirable. This is so for two reasons, (1) that any change of objective or eyepiece results in alteration of luminosity of the field of view, and (2) that preparations of varying density are better seen when the light can be adjusted to suit them. The first condition is not generally a consideration in photo-micrography as exposure is altered to suit such changes. The second point is of importance and can be met in several ways. The easiest and most satisfactory is to provide some semi-silvered or semi-platinised pieces of glass of various densities. These are not difficult to prepare, and a method is described in the Appendix to this volume. Another method is to use a neutral-tint wedge, or alternatively one or more pieces of neutral glass (obtainable from Messrs. Hetley & Son, Soho Square, London). A convenient Neutral Wedge Light Moderator is made by R. & J. Beck, Ltd. (Fig. 37). This consists of two neutral-tint wedges which are geared together so that they move in opposite directions, thus forming a variable light screen. They are mounted on a stand adjustable for height so that they can be conveniently placed in any desired position in the illuminating beam. There is a great advantage in using a double wedge such as this, the intensity over the area of overlap being even and equal, whereas with a single wedge the intensity must be unequal over the illuminated area.

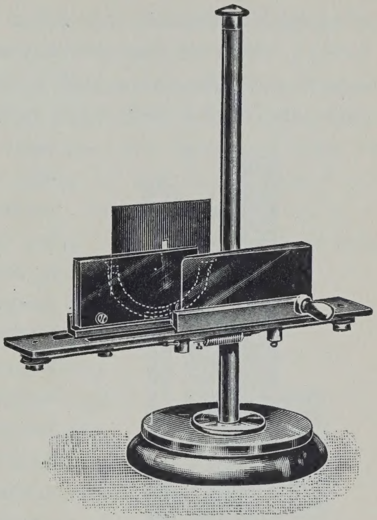


FIG. 37.—Neutral Wedge Light Moderator. (R. & J. Beck, Ltd.)

Oil Lamps.—If the length of exposure necessary is no particular objection, a good oil lamp, carefully chosen and well constructed, will answer many purposes. The lamp should be either of the type in which the wick is broad and flat, or with a circular burner of the Argand pattern. If of the former variety it should have a wick at least one inch broad and should be so arranged that the lamp can be turned either with the broad side or with the edge of the flame towards the microscope. The type of microscope lamp sold by opticians is often not the most suitable; they are generally small in the body, and altogether not solid or substantial enough in construction. One should be chosen with a vertical adjustment for centring the light. This movement is usually obtained by carrying the oil reservoir on a bracket which slides up and down on a vertical rod rising from the stand, and which can be clamped in any position (Fig. 38). The writers, however, have used an oil lamp of simple construction which has been found in every way satisfactory. The oil reservoir is a metal chamber about six inches square and one and a half inches deep. The burner is a circular one of the best make procurable, and the light is sufficient for any work except when the use of oil-immersion objectives is necessary. No provision is made for vertical adjustment, as with such a large source of light it can be placed in position in relation to the microscope once and for all. With all oil lamps the heat emitted is considerable ; so the lamp should be placed as far from the microscope as possible, and it is always advisable to interpose between the microscope and lamp, but as near the latter as possible, a blackened metal diaphragm of fairly large size with a hole in it about three-quarters of an inch in diameter.

The petroleum used should be of the best quality, and should have from 2½ to 3 per cent., by weight, of camphor dissolved in it. The wick should be evenly trimmed and thoroughly dried before it is inserted in the wick holder. All parts of the lamp should be kept clean, as on this depends the efficiency of the light and freedom from smell. The glass chimney should preferably be of Jena or some similar glass, to lessen the risk of fracture from changes of temperature.

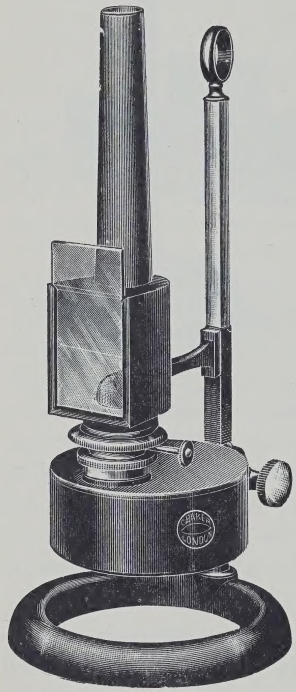


Fig. 38.—Oil lamp.
(C. Baker.)

The chief objection to an oil lamp is that it necessitates prolonged exposures, especially with magnifications exceeding 500 diameters ; although some of the best photo-micrographers have obtained excellent results and recommend it for all classes of work.

The duplex form of burner is to be avoided, as, although a higher degree of incandescence is obtained owing to the close proximity of one flame to the other, the inevitable dark space that occurs between the two makes its use difficult where an image of the radiant is projected in the object-plane.

Coal Gas.—The Welsbach or incandescent gas light, although it has a higher illuminating power than an oil lamp, suffers from a considerable disadvantage. In using this source of light for microscope illumination, the image of the incandescent gas mantle becomes painfully evident. The spectroscopic value of the light is such that when using colour screens (which in most cases are necessary) the light is so deficient in certain parts of the spectrum, that it becomes in effect a very weak source. Where no other illuminant is available, a simple and efficient modification of the incandescent gas lamp may be obtained from Messrs. Hawksley & Sons, Wigmore Street, London. The light is contained in a metal tube and is supported conveniently for miscroscopic use (Fig. 39). It consists of a small atmospheric burner similar to that used for acetylene gas, and a high temperature is attained. The flame impinges on to the end of a rolled-up piece of flexible incandescent mantle. This is made about the size of a cigarette, and is contained in a silica tube which projects behind the lamp and is held by a clamping screw as shown. When the gas is alight the mantle is pushed out of the silica tube for about 5 mm., and is then brought into the flame by means of a screw provided for that purpose. Some little adjustment is necessary, but when this is carried out a very useful form of light results. Should the protruding piece of mantle require renewing this can be done by pushing forward another short length, a process that can be repeated many times. Where coal gas is not available, the lamp can be run by means of high-pressure methylated spirit.

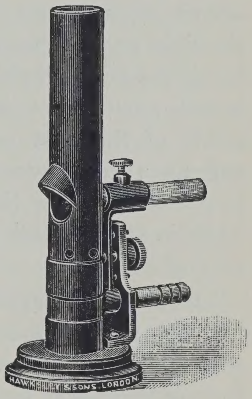


Fig. 39.—Modified Incandescent Lamp. (Hawksley and Sons.)

Acetylene.—Of the less powerful sources of light, acetylene gas has some advantages; it is brilliant and yet the flame is comparatively cool. Acetylene, or to give it its scientific name, ethine (C_2H_2), is a gaseous compound of twenty-four parts by weight of carbon with two parts by weight of hydrogen. It is evolved by the action of water upon calcium carbide, which causes a double decomposition to take place, the oxygen of the water combining with the calcium and forming lime, which remains as a residue, while the hydrogen unites with the carbon of the carbide to form acetylene.

The light given out on burning the gas is very actinic, and spectroscopically the nearest in value to sunlight; there is a very even distribution of light throughout its spectrum—no colour in particular predominating. The consequence is that the light appears very white. It has the additional advantage for photo-micrographic work that the amount of radiant heat is not very great; hence expansion of the apparatus, as the result of the heat emitted, is not a disturbing factor. The exposures necessary with it will be found to be about one-seventh to one-tenth of the time required with the oil lamp. The light itself is steady, and its luminosity is uniform throughout the area of the flame.

Lime-light.—Oxy-hydrogen light or lime-light is produced by raising the temperature of the surface of a cylinder of lime to a white heat by means of a flame produced by the combustion of hydrogen and oxygen. The necessary gases are supplied compressed into steel cylinders. Pure hydrogen can be replaced by coal-gas.

The mechanical contrivance that is used for combining and burning the gases and bringing them into contact with the lime surface is known as a lime-light jet. There are two types of jet, known respectively as the 'mixed jet,' and the 'blow-through jet.'

In the mixed jet, which is the more powerful, the gases, hydrogen and oxygen, are intimately mixed in proper proportions in a chamber, before they issue from the orifice of the jet and impinge on the lime.

In the blow-through jet the ordinary house gas is used directly from any convenient source of supply, and the oxygen alone is under pressure. It has a larger area of incandescence than the mixed jet, but its illuminating power is not so high.

Lime-light as a radiant has many excellent qualities. The source of light is constant, and in the mixed jet is not of large area. It is obtainable in out-of-the-way places, where electric light is not available. It is cheap and easy to work, and free from danger when carefully used and its principles understood.

If reference is made to the illustration of a mixed jet (Fig. 40), it will be seen that it is constructed as follows:

The hydrogen is conducted in at one tube, usually on the left side, and the oxygen passes through the other; the gases then pass into a chamber which is packed with iron gauze or some similar material, so that they there become thoroughly mixed. Then they pass out through the nozzle and impinge on to the lime which is placed on the pin in front of the nozzle. The gases, which are contained in steel cylinders, must be under equal pressure, and on each cylinder, therefore, some form of regulator must be placed, so that this pressure may be regulated and controlled. The efficiency of the mixed jet depends on the proper mixing of the gases, and on the maintenance of equality and regularity of pressure.

In working the jet, the hydrogen, or compressed coal-gas, must be allowed to pass through first, and should then be lighted at the orifice of the jet. The flame should be sufficiently high to rise well above the top of the lime cylinder, but should not be high enough to flare. The oxygen should then be carefully turned on so that only a small pressure of gas comes through at the start. The immediate effect will be for the hydrogen flame to go down; some more hydrogen should then be let in and next a further supply of oxygen, each being successively turned on in small quantities until a hissing or roaring sound is heard. The point at which the best light is obtained is just short of this condition in which hissing occurs.

The distance of the lime from the orifice of the jet is of great importance, and it should be carefully moved away from the jet until no black spot on the lime can be seen. This is perhaps somewhat difficult to observe directly, but any piece of glass or the surface of a condenser that may be in use at the time will act as a reflector if temporarily placed a short distance in front of the lime.

The blow-through jet is somewhat more simple to use. The tube through which the coal-gas is conducted may be connected to a house supply. This should, when lighted up, give a flame from an inch to an inch and a half higher than the top of the lime cylinder. The oxygen should carefully be allowed to pass in from the cylinder, and very much the same condition of affairs will be noted as in the mixed jet, excepting that the adjustment of the oxygen is the only one of importance. It should be allowed to pass until there is a distinct falling off in the light. If there is any excess of coal-gas it will show itself by the flame extending above the top of the lime, the coal-gas must then be gently turned off until only a slight excess is apparent.

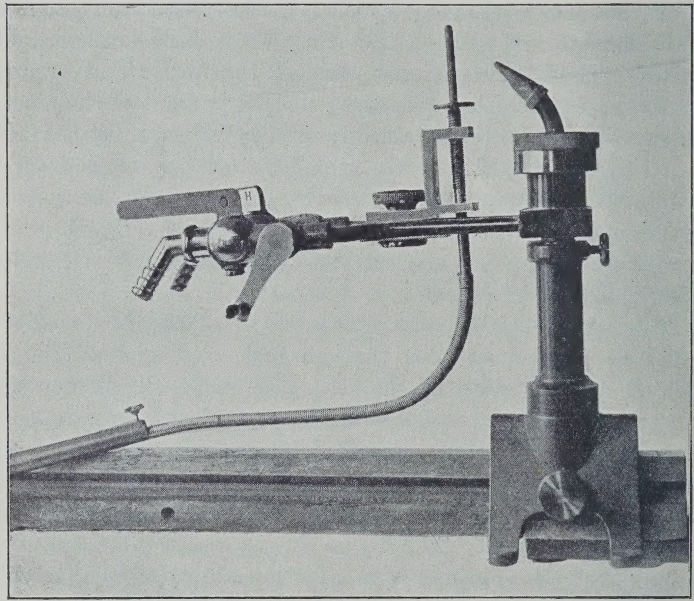


Fig. 40.—Lime-light Jet on Saddle-stand.

The selection of the limes for either jet is of some importance. For the mixed jet a hard form of lime is necessary, as both gases work under pressure; and as the temperature is so high, pitting of the surface of the lime quickly takes place. This is to some extent overcome by turning the lime at intervals of five or ten minutes—the time depending largely on the pressure of the gas—so that a fresh surface of the lime is exposed to the action of the jet.

In the case of the blow-through jet, a softer form of lime can be used; as the surface of incandescence is larger and one gas only is under pressure, pitting does not so quickly occur.

Fig. 40 shows a mixed jet to which a special cut-off is fitted, so that the light can be turned up or down without altering the relative adjustment of the gas or oxygen taps. This is a great convenience in photo-micrography as the light is only required for short periods.

Electrical Illuminants.—It is now so general for a supply of current to be available that, both on the score of convenience and of economy, one of the many forms of electric light is to be preferred.

Among those suitable for photo-micrography are the Pointolite lamp, the mercury-vapour lamp, and the electric arc. Ordinary incandescent metallic filament lamps of the vacuum type are unsuited for this purpose. In these the filament is far too thin, and owing to its length, the source of light is not sufficiently concentrated. Even though a diffusing medium, such as ground-glass, be used between the light and the microscope it does not answer well, for when the initial source is of low intensity, as it is in such filament lamps, the loss of light becomes a really serious factor.

The Ediswan Pointolite Electric Lamp (Fig. 41) is the most useful illuminant at present available for the microscopist. It furnishes a light-source of small size and of high and uniform intrinsic brilliancy. Its cost is its greatest objection, but the life of the lamps appears to be longer than when they were first introduced. Full particulars can be obtained from the Ediswan Company as to price and constructional details. The source of light is a small tungsten sphere which is raised to a high temperature short of the melting-point of the metal. This sphere is enclosed in a glass globe, such as ordinarily used for gas-filled electric lamps of small size, so that a condensing lens may conveniently be brought near to the light-source. The lamps are made in several sizes, but the most suitable for photo-micrography is 100 c.p. A special form of starting resistance is required which is adjustable for any lighting circuit voltage, but a cheaper form of non-adjustable resistance is now available. The makers supply a form of carrier for the lamp, which is practically the same as that used for lime-light apparatus. If it is adopted the reflector should be removed and only the direct light from the illuminant allowed to reach the microscope. A point that is of some importance is to place the lamp so that movement of the tungsten ball, which occurs each time the light is switched on or off, is along the axis of the microscope. This can be done by arranging the lamp so that the heating coil inside the bulb is nearly in alignment with this axis. The life of a lamp is somewhat increased, as with all incandescent electric lamps, by using a lamp made for a rather higher voltage than that of the circuit (e.g. 110-volt lamp on 100-volt circuit). In the case of the Pointolite this is achieved by suitably altering the standard resistance, the lamps as supplied being the same whatever the voltage of the supply circuit may be. The standard resistance is provided with plug-in sockets covering from 100 to 240 volts in steps to suit well-known supply voltages. It is therefore advisable to set the resistance at the next point higher than that indicated. The plug should, for example, be put in the 110-volt socket when the lamp is to be used on a 100-volt circuit. This results in a slight drop in brilliancy, but not sufficient to make a material difference in the length of exposure necessary. The lamp is now obtainable for either continuous or alternating current.

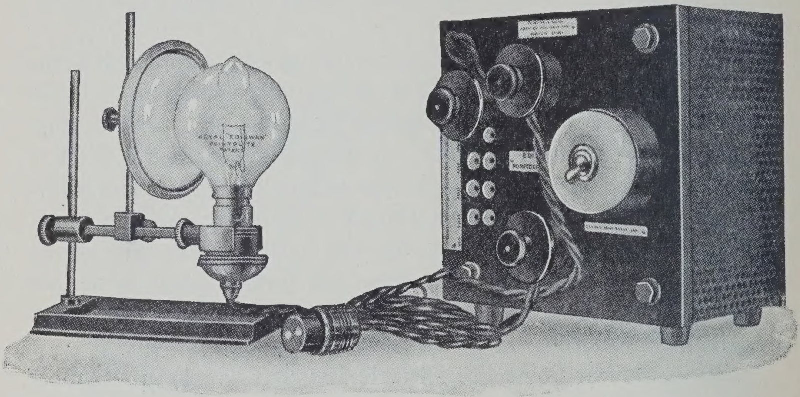


FIG. 41.—Pointolite Lamp and Resistance. (Ediswan Electric Co., Ltd.)

The alternating-current lamp has two tungsten spheres, both of which become incandescent. There are therefore two light sources and this is not desirable for microscopic work. To obviate the optical difficulties ensuing, the bulb should be rotated in its holder so that these two spheres are in line with the optic axis, that is, so that one is in front of the other and obstructs the light from the rear sphere. Loss of light does, of course, result, but this is of little importance when compared with the improved conditions obtained.

Mercury-vapour Lamp.—One of the forms of electric light likely to prove valuable in photo-micrographic work, and in fact in all microscope illumination, belongs to the type of which the mercury-vapour lamp is an example.

In this method of light-production we have what is really an arc light, but with the very important difference, that in the mercury-vapour lamp the gaseous arc *itself* constitutes the source of light, instead of one or both of the solid poles as in the carbon arc. Further, the current necessary to maintain a mercury-vapour lamp is smaller than that required to maintain any carbon arc. The mercury vapour which constitutes the source of light is enclosed in a glass, or preferably a quartz tube, and so is not subject to atmospheric influence; it may be described as an hermetically sealed mercury arc.

The production of light depends on the same essential conditions as in the arc light. The mercury, however, constitutes the poles between which the arc itself plays. The mercury is vaporised at the negative pole, and the vapour becomes highly incandescent as the result of its molecular activity and not, as in the arc and other electric lights, as the result of rise of temperature. Spectroscopically, the light consists mainly of three bright lines—one each in the yellow, green, and blue-violet regions. It also has a very extended ultra-violet spectrum but for the purpose under consideration this is not of moment. It is only necessary to screen off the colours which are not required, with suitable colour screens, by a method to be described later, in order to obtain a visible light source which is truly monochromatic. Even without any colour-screen it is more efficient optically than other illuminants, since owing to the absence of red radiations the mean wave-length is less. Consequently, the defining and resolving power of any microscope objective is appreciably increased.

The type of mercury-vapour lamp supplied commercially for ordinary photographic purposes is not suitable for microscopic work, as the containing-tube is far too large. One of smaller size and having a tube of much less sectional area has proved to be satisfactory. The current-consumption is small, so that it is a source of light that can be used with safety on any house-lighting circuit, current being obtained from an ordinary wall-plug or lamp-socket. It has the additional and by no means unimportant advantage, that there is practically no radiant heat, hence the lamp can, if necessary, be run in very much closer proximity to the microscope than is possible with any other light-source yet described. No water-cell to absorb heat need be placed between the illuminant and the microscope.

As the mercury is enclosed in a tube, exhausted to such a degree that its vacuum is considerably higher than that of an ordinary incandescent electric lamp, no consumption of the mercury takes place. The mercury is volatilised, but, when the current is switched off, it is at once recondensed and no loss of mercury occurs. The lamps usually have a long life, excluding the risk of accidental breakage of the tube, and their visible light efficiency goes down but little even after running for a thousand hours. This is a characteristic by no means common in electric lamps other than the ordinary arc, for with most of them their efficiency tends to decrease rapidly after they have run for a few hundred hours.

There are two types of these lamps available at present, one in which a permanent vacuum is maintained, and the other in which the breaking of the mercury column results in the formation of the arc. The former is easily obtained commercially, but has the disadvantage that if the vacuum is disturbed in any way the tube must be opened, re-exhausted and re-sealed, a process involving considerable expense. The second type is to be preferred and can be obtained from Messrs. Kelvin, Bottomley & Baird. The burner consists of a transparent fused quartz container which is not exhausted, but which works under atmospheric pressure; thus the difficulty of making vacuum-tight seals in quartz is obviated. When the lamp is not in operation the burner is filled with mercury; no tilting mechanism is necessary, the lamp starting automatically when current is switched on.

As a light-source the mercury lamp has much to recommend it, but it can hardly be regarded as one to rely on exclusively. It is in fact suitable for visual rather than for photographic work. Although total energy output may be large, intrinsic brilliancy is not high. A small intense light-source is desirable in photo-micrography, whereas a mercury-vapour lamp is large in area rather than of high intensity. For some purposes, however, its use is an advantage as monochromatic light of great purity can be obtained. For the photography of faintly stained specimens it is unrivalled and in dark-ground work if long exposures are possible it gives results that can hardly be equalled by any other method.

The burner is shown in Plate II, Fig. 1.

The quartz container comprises a lighting tube with mercury reservoirs at each end, which serve as the electrodes, into which the conducting wires are sealed through tubular projections.

A short limb projects from the negative side of the tube, which is surrounded by a detachable heating coil. The function of the limb and heating coil is to start up the lamp. The heating coil is connected up in series with the burner tube, through the auxiliary electrode. When the current is switched on the coil becomes red hot, the mercury boils in the limb causing rupture of the mercury column, which starts up the arc. In some recent forms a different type of heating coil is provided, but this does not involve any alteration of principle.

As the arc extends along the tube the mercury is forced back into the reservoir through the constriction, until it makes contact with an electrode in the cut-out device.

When contact is thus established, the heating coil is automatically cut out of circuit. Should the arc for any reason be extinguished, the mercury will fall back and refill the tube, and thus, by breaking contact, cause the heating coil to come into action again.

The burner tube is mounted in a simple frame, carried on a rod. All that is necessary is to hang the frame in two suspension hooks, and connect up the leads from the positive and negative electrodes to the terminals.

Once the lamp is properly installed it is impossible to reverse polarity, as the suspension hooks are arranged so that the burner will only fit in one direction, that is, with the positive electrode under the positive terminal.

A resistance must be provided suitable for the voltage of the supply circuit. The actual starting current is about 5 amperes, but this is reduced in running to about 3 amperes. Polarity must be ascertained and the connections made as indicated by the makers.

The actual form of the mercury arc in its quartz tube is shown in Plate II, Fig. 2. This image was photographed by means of a pin-hole camera, the pin-hole taking the place of the lens.

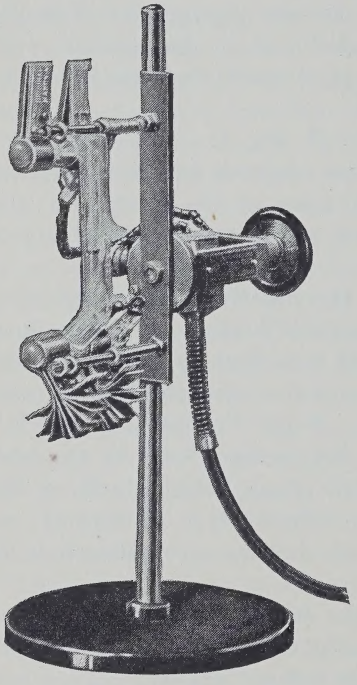


Fig. 42.

Vacuum-type lamps are made by the British Hanovia Quartz Lamp Co., and by the Thermal Syndicate, Ltd., either of which is suitable for microscopical work. The Hanovia Co. sell two forms for direct current, one known as the "Scientific Lamp" and the other as the "Point Source Lamp" and types are also available for alternating current. The Scientific type is seen in Fig. 42 and its chief advantage is that the burner provides a small but intense end-on point source of light. This largely obviates the disadvantage of a long light-source in a narrow tube and does reduce the chance of flicker. At low potentials the arc or electric discharge fills the whole cross-section of the tube, but as the potential increases the arc diminishes to a diameter of about 4 mm. The light is constant and will run for long periods without interruption. The lighting of the lamp is effected by tilting the burner.

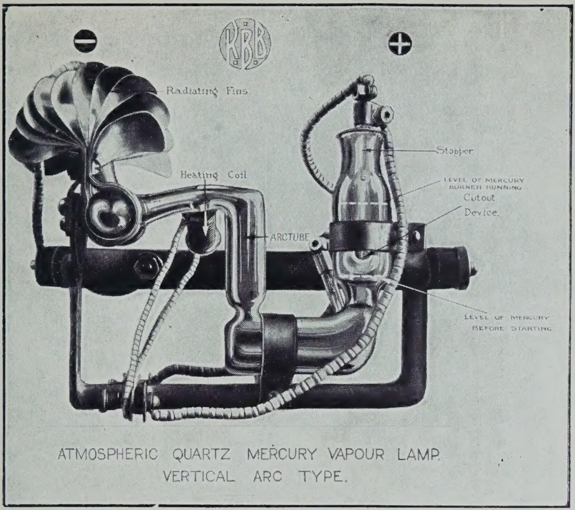

−
KBB
+
Radiating Fins.
Heating Coil
ARCTUBE
Stopper.
LEVEL OF MERCURY BURNER RUNNING.
Cutout Device.
LEVEL OF MERCURY BEFORE STARTING.
ATMOSPHERIC QUARTZ MERCURY VAPOUR LAMP.
VERTICAL ARC TYPE.

FIG. 1.—Burner.

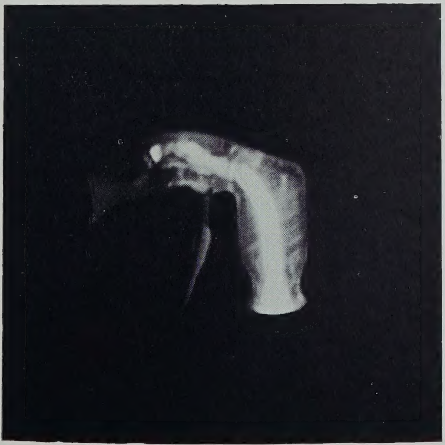


FIG. 2.—Form of Mercury Arc.
Mercury-vapour Lamp.

The ‘Point Source Lamp’ is provided with one tungsten and one mercury electrode so that, although the source of light is small, higher intensity can be attained.

The Thermal Syndicate supply a ‘Mercury Discharge Burner’ which is designed for microscope illumination. It is self-starting, contains only a small quantity of mercury and can be used in any position. It operates on A.C. mains through a transformer but is of low current capacity, so that it is more useful in visual work than in photo-micrography.

The Electric Arc.—Of the high-power illuminants available the electric arc is, both theoretically and practically, almost ideal. From the point of view of practicability it is probably the most convenient, as every laboratory and many houses have a suitable supply of electricity; and it has the advantage that the source of light is small and of uniform intensity.

The production of an electric arc is dependent upon the fact that if an electric current of suitable voltage is allowed to pass between two carbon points which are slightly separated, one or both of these points becomes a highly incandescent source of light.

There are two methods of producing and maintaining an arc light; one, in which some automatic method is used of regulating the distance between the carbon points, and the other which depends on a simple arrangement whereby they are adjusted by hand.

Let us for a moment consider the essential conditions for producing an arc light with continuous current. Most main sources of electrical energy are such that the current is supplied at from 100 to 250 volts. An electric arc requires only from 40 to 50 volts; so that some means must be provided of absorbing the difference between the voltage of the supply and the potential difference required between the carbons. This is accomplished by putting a resistance in series with the lamp—that is, an arrangement in which some of the electrical energy is expended in producing heat. The term ‘in series’ means that the lamp and resistance-coil are arranged so that the current passes successively through each.

To start an arc it is essential that the two carbons should momentarily touch. Be the distance between the carbons ever so small, a current of electricity of low voltage will not pass between them unless this preliminary contact takes place. On making contact, some small portion of the extremity of the carbons is volatilised ; on parting them again, the volatilised carbon acts as a conductor, and the electricity passes across the gap ; in so doing, it raises the points of both carbons to a condition of incandescence.

It is the practice in continuous current arc lamps to place the positive carbon—that is, the one that is connected to the positive wire coming from the electrical supply—in the uppermost position, the negative carbon being placed in the lower holder of the lamp. When an arc is started, it does not at first burn steadily, nor is the source of light a constant or regular one. This is due to the fact that, until what is known as a ‘ crater ’ is formed on the tip of the positive carbon, the light does not settle down and become steady. This crater is really the source of light, and in microscopical work it alone is used, the negative carbon being so placed that the light from it does not reach the microscope.

The ends of both carbons are generally tapered off, and in course of burning the tips tend to keep this shape. The crater on the positive carbon should be of such diameter that it is well confined within the limits of the tapered point—there should be no tendency for the white-hot portion of the crater to go over the edge of the carbon. This will never happen if the size of the carbons is correct, but the larger the current, the larger must be the size of the carbons used. When the current is too large for the carbons the arc begins to hiss, and the luminous point spreads and extends over the edge of the crater. To decrease the chance of the luminous point altering its proper position on the end of the positive carbon, the latter is always ‘ cored ’—that is, it has a hole through its centre which is filled with softer carbon than the outer portion. This softer core vaporises more rapidly than the surrounding harder carbon, and the crater therefore tends to remain in its proper position.

With a continuous-current arc in which the carbons are coaxial the position of the carbons should be as shown (Fig. 43). The positive carbon should be set somewhat behind the negative one so that the crater tends to form towards the front and facing the direction in which the light is required. The carbons should be placed so that their front surfaces are approximately in a straight line. They should also be inclined at an angle of about 27° to the vertical; at this angle two essential conditions are secured—the greatest amount of light is thrown forward from the crater of the positive carbon, and practically the whole of the light from the negative is thrown to the back, and eliminated as a second and harmful source of light.

There is a critical length of arc at which the maximum light is obtained, and the greatest efficiency and constancy secured. With a current of, say, ten amperes, the best length of arc will be found to be from three to four millimetres; but with any hand-feed method of adjustment this can be determined by experiment. The arc should not be so short that hissing occurs, and should not be so long that there is any variation in the position of the luminous crater on the extremity of the positive carbon.

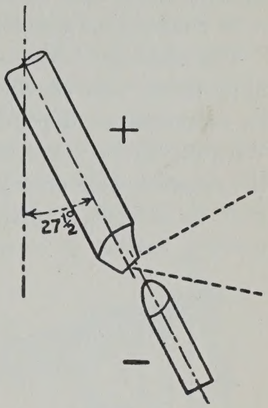


Fig. 43.—Correct Position of Electric-arc Carbons.

The sizes of carbons that should be used may be approximately as follows:

Current in amperes.	+ (Cored) mm.	− (Solid) mm.
5	7	5
10	9	6
15	10	7

Observation of the form of the negative carbon is useful in determining whether the lamp is burning properly and with a correct current supply. Under proper conditions it should have a regular cone-shaped head pointing towards the crater on the positive carbon (Fig. 44 *a*). If the current is too large, the tip of the negative carbon will become blunt and rounded (Fig. 44 *b*); and if too small, a mushroom-shaped excrescence forms on its tip, and the lamp burns unsteadily (Fig. 44 *c*). In the former case the resistance in series with the lamp requires increasing, so that the current is decreased in amount, while in the latter case the resistance should be decreased, a corresponding increase of current resulting. The distance between the carbons must also be modified—either lengthened or shortened—to conform to the altered conditions.

The distance between the two carbon points is of primary importance, and on the careful regulation of this distance and its maintenance depends the constancy of the source of light. With continuous current the positive pole is the light-producer; the negative pole only produces half as much light as the other, and, as already explained, this should not be utilised. The positive carbon is consumed at a rate that is approximately double the rate of consumption of the negative. To obviate the difficulty due to this difference of consumption, the sectional area of the positive carbon is made twice that of the negative one, so that the reduction of their lengths is practically constant. With alternating current the rate of consumption of the two carbons is equal. The carbon poles are equally incandescent and, consequently, only one-half of the light produced can be used. Any method of automatic regulation is less certain than with continuous current, but recent experience has convinced us that an alternating-current arc can be a satisfactory light source. Certain conditions must be complied with to secure a satisfactory result and will be referred to later in this chapter. This matter is now of greater importance as so

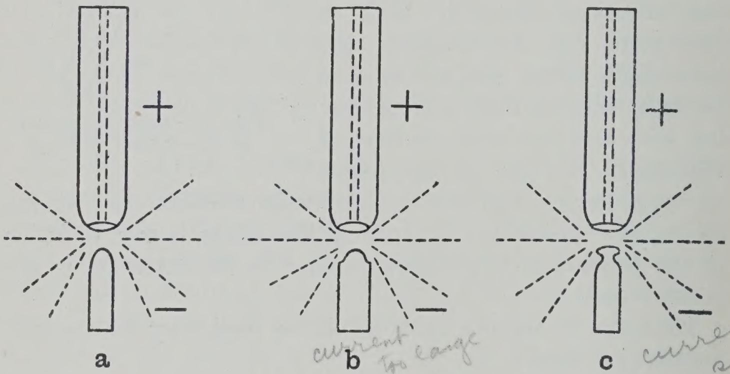


FIG. 44.—Appearance of Electric-arc Carbons under Different Conditions.

many electrical supply concerns have changed over from continuous to alternating current.

A convenient current consumption for a continuous current arc is from five to ten amperes. It should be understood that increase of current consumption does not result in any increase of incandescence of the luminous crater. The carbon is already at the point of volatilisation, so that increase of current cannot raise it to a higher state of incandescence.

The simplest method of adjusting the length of the arc and keeping it constant both in position and condition is by the use of a hand-feed arrangement. Any exact method of feeding the carbons may be adopted, and Fig. 45 shows a form of mechanism. Here the two clamps holding the carbons are supported in a centre casting, and are actuated by an ordinary rack-and-pinion motion, this latter being controlled by the milled head B.

The vertical adjustment of the arc is controlled by screw D and lateral adjustment by the milled head C. The milled head A allows movement of the positive carbon-holder so that the positive crater is caused to form towards the front of the lamp. Whatever arrangement is decided on, a complete absence of shake, and no unsteadiness of movement or displacement other than in the direction desired are essential conditions.

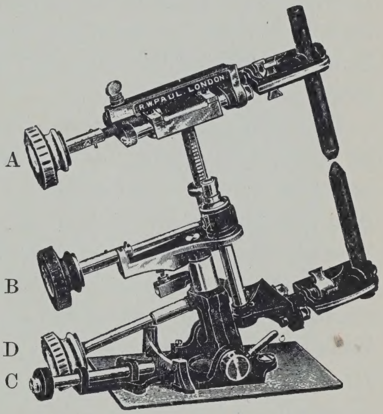


Fig. 45.—Hand-feed Electric-arc Lamp.

The particular means by which the carbons are moved in relation to one another is not of great importance, provided that both are moving at the same rate and that no lateral movement occurs. There should be a means of moving the carbons so that without alteration in their relative position, the arc is itself moved as a whole. It will be found in practice that any adjustment of the arc, whether for position or length, is only necessary at intervals of one to two minutes.

In any ordinary exposure an adjustment made before the exposure is commenced will ensure the arc remaining in its proper condition for a sufficient length of time. When the lamp is started no attempt should be made to take a photograph with it for a minute or two, as probably owing to the fact that there is a considerable amount of occluded air in the carbons, the light does not immediately become steady.

It does not come within the province of this work to discuss the construction of automatic arc-lamps in detail, so that only two types will be described.

In these the carbons are inclined or at right angles to one another, the object being to project the light from the positive carbon directly towards the microscope, the negative carbon being so disposed that the light it emits cannot reach the microscope.

This arrangement requires that the positive carbon should be small in diameter for the current used, so that the crater occupies a large portion of the end of the carbon. The tendency is for the arc to take the shortest course across the gap between the two carbons, and consequently it may happen that more light is projected behind than in the direction of the microscope. The advantage is that, after the necessary preliminary adjustment, the positive crater always remains central, since the consumption of the positive carbon takes place in the direction of the optical axis.

It must be recognised that any form of automatic regulation, unless it is of the best construction, is worse than useless. In the hand-feed arrangement there is no mechanism to get out of order, but in the automatic the whole efficiency of the apparatus depends on an immediate response to small changes in the current flowing through the lamp.

Usually an automatic lamp is intermittent in action, and its mechanism is only brought into play as the result of some lengthening of the arc and consequent alteration in the amount of current; the change required to set the carbons in motion depends entirely on its sensitiveness. It is therefore difficult to ensure the constancy and accuracy obtained by a simple hand-feed apparatus.

The type made by Messrs. Carl Zeiss is seen in Fig. 46. It is mounted in a casing which is supported on three points, these being kept in position on the bench by the hole, slot and plane method (Fig. 47). Two of the three supporting points are on the end of long screws as shown in Fig. 46, so that the lamp may be adjusted in height. The ends of the screws are pointed, one resting in a conical hole, the second in a V-shaped slot and the other on a plane surface, the relation of these being shown in Fig. 47. The positive carbon is horizontal, and the source of light therefore remains in a constant position. The negative carbon slopes forward to reduce the possibility of obstruction of the light coming from the positive carbon. The feed is automatic and takes place at intervals as the arc lengthens. If carbons of good quality are used the lamp works with remarkable steadiness, but some attention is necessary to see that the relative position of the two carbons is maintained. To provide for this the positive carbon has an adjustment so that it may be moved forward or backward as may appear necessary. A small observing window is provided at the side of the lamp so that the state of affairs can be seen, the direction of observation being such that the points of the carbons are seen from

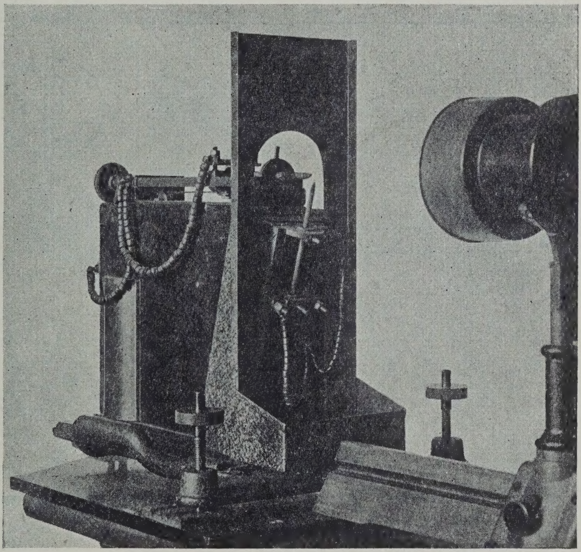


Fig. 46.—Arrangement of Carbons in Zeiss Arc Lamp with Automatic Control.

the side. Reference to Plate III will show how the carbon points should appear (A1) when the adjustment is correct but the lamp is not running, A2 showing the side view when the lamp is running, A3 the image of the arc as projected forward by any collecting-lens. It will be seen that under these conditions the end of the positive carbon is evenly luminous and a circular uniform disc of light results. B1 shows the state of affairs when the positive carbon is too far forward with the corresponding change in the images as seen in B2 and B3. These show not only that there is considerable loss of light, but that the luminous area is irregular in shape and is altered in position. C1 shows the reverse state of affairs, the negative carbon being too high. This may be remedied by moving the positive carbon forward and restarting the arc. C2 and C3 show respectively the side and front view of the carbons when in this condition. These figures indicate the adjustments that must be made to ensure satisfactory running with any right-angled carbon arc, whether hand-feed or automatic, and they indicate the necessity for an adjustment to obtain accurate setting of the carbons in relation to one another.

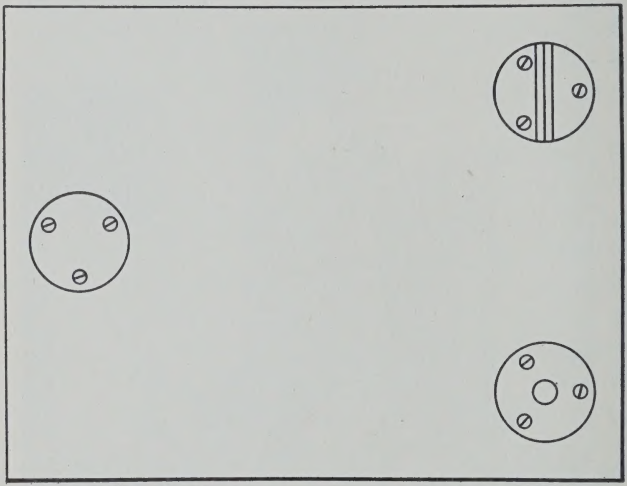


Fig. 47.

The automatic arc lamp made by Leitz is in general appearance similar to Fig. 48. In this type the carbons are fed by means of a clockwork mechanism. The clock is automatically wound up when the carbon holders are moved backwards to replace the carbons. The feed is continuous and the clock must be regulated by the contrivance provided for that purpose to ensure a proper rate of feed. The relative size of carbons advised by the makers is essential to ensure satisfactory running. A hand-feed lamp of similar design is also supplied, and either pattern can be used with alternating or direct current.

It will be advisable to give a brief description of the method of connecting up a continuous current arc lamp to the source of electrical supply. The conducting wires will require to be thicker than those used for ordinary lighting purposes, and should have fuses, or what are known technically as ‘cut-outs,’ exactly suited to the current to be used.

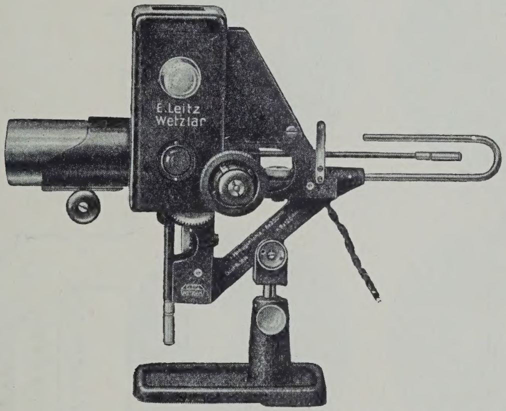


Fig. 48.—Automatic Arc Lamp. (E. Leitz.)

Fig. 49 indicates the method of connecting up the lamp. The wires are brought to the points on the board, respectively positive and negative, as marked. They then pass to the safety fuses A, and thence to B, a double-pole switch for making and breaking the circuit. One wire is continued from either terminal—it matters not which—to one end of the resistance coil C. The other terminal of the resistance is connected up directly to the proper terminal of the lamp. The other wire from the double-pole switch is then connected directly from the switch to the other terminal of the lamp, it does not connect to the resistance.

When the lamp is to be started, if it be automatic, it is only necessary to switch on the current, and the arc is at once made. With the hand-feed lamp, after the current is switched on the two carbons have to be brought together momentarily and drawn apart, as already explained, when the lamp will immediately light.

If desired, an ammeter and voltmeter can be connected up in the circuit. The ammeter is arranged so that the whole of the current passes through it in the same way as through the resistance, i.e. ‘in series.’ The voltmeter must, however, be connected up as a shunt-circuit, so that it indicates the difference of potential on the terminals of the lamp. These electrical instruments are by no means necessary, although at times they are a convenience; but if all the arrangements are made by a reliable and competent electrician, as they should be, there will be little need for their use.

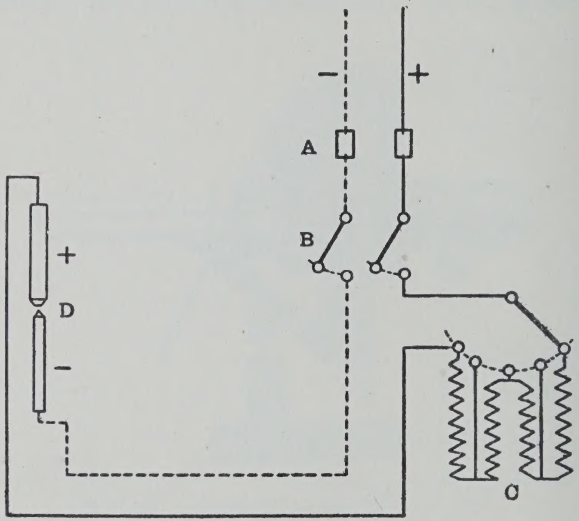
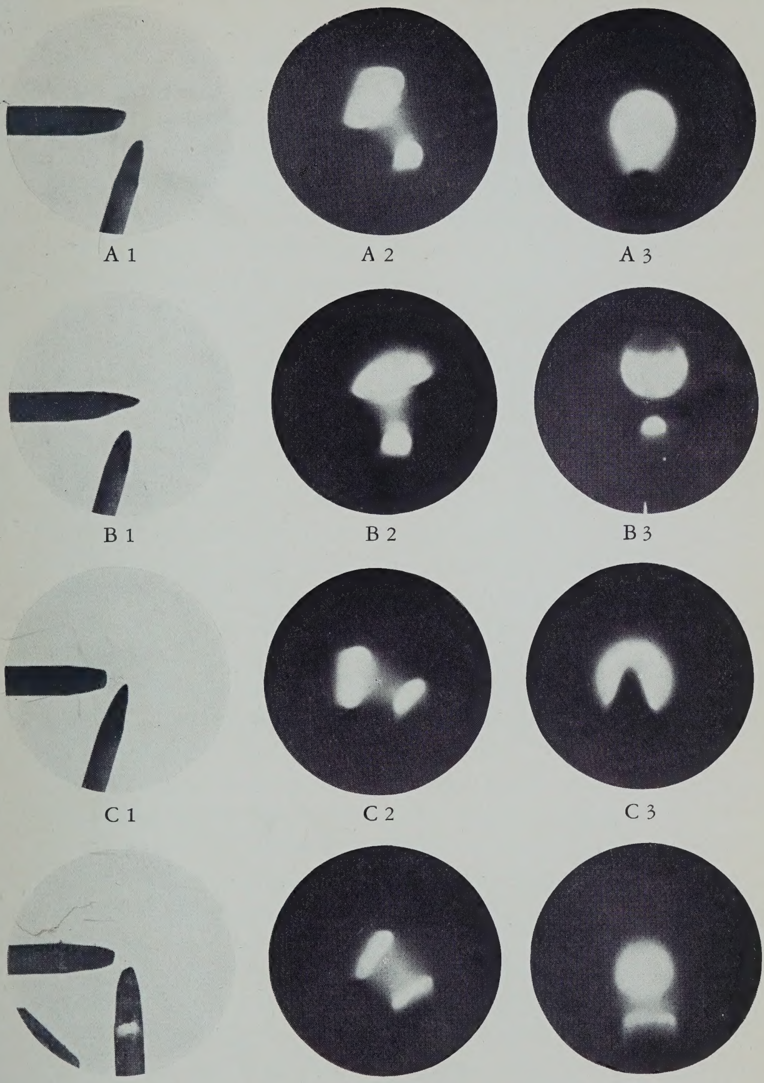


Fig. 49.—Method of connecting up Electric-arc Lamp.

The connections for an alternating-current arc are the same as for continuous or direct current. The only difference is that polarity need not be considered, there are neither positive nor

PLATE III



A 1 A 2 A 3

B 1 B 2 B 3

C 1 C 2 C 3

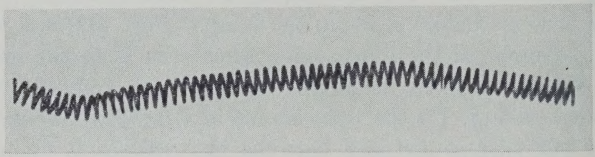
D 1 D 2 D 3

Adjustment of Carbons in Arc-Lamp.

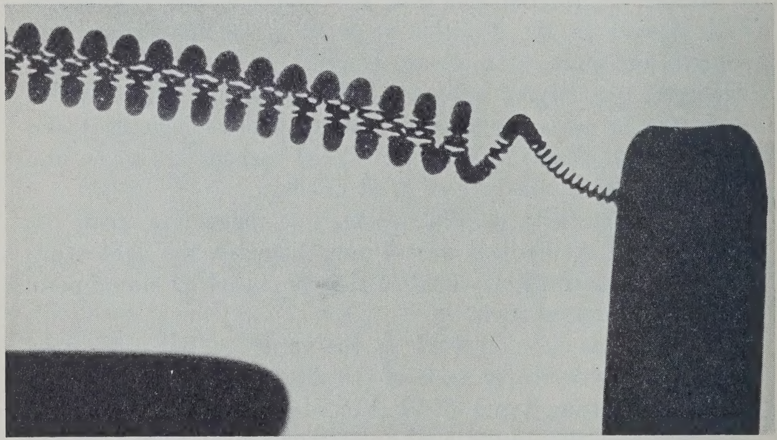
negative poles. If the lamp is of the hand-feed variety, with an equal rate of movement to each carbon, the carbons must be of the same size. If the rate of feed is dissimilar the size of carbons must be adjusted accordingly. In any case the diameter of the carbons must be as small as possible, so that the luminous area occupies the whole of the carbon point and thus has less opportunity to wander. Plate III, Figs. D1 to D3 demonstrate the effect of carbon adjustment in the alternating current arc. Fig. D1 shows the correct relative position of the carbons before starting the lamp. Figs. D2 and 3 show the appearance of the light-source when seen from the side and from the direction in which the light is projected. It will be seen that in Fig. D3 the light-source is a uniform circular disc, and if the correct distance between the carbons is maintained this disc remains constant. The light from the vertical carbon is thrown backwards and does not interfere, one light-source only is seen in the direction required. The illustrations will suggest the most desirable arc length. As both carbons emit the same quantity of light one-half is wasted. It is therefore necessary to use a larger current to produce enough light and to ensure steadiness in running. Cored carbons are to be preferred and they must be of good quality.

The arc shown in the illustrations was consuming about ten amperes and the carbons were 4 mm. diameter, the light available was approximately equal to that produced by six amperes with continuous current.

A lamp has been evolved as the result of our laboratory experience, to overcome some of the disadvantages to be found in many existing types of electric filament lamps. From a microscopical standpoint opal and ground-glass globes are not ideal; the light emitted is increased in mean wave-length on passing through most opal glasses, and even initial efficiency is not maintained for any great length of time. The lamp now described is similar in principle to one made by Mr. J. W. Gordon so long ago as 1908. The method is to use a glass rod to transmit the light from the illuminant to the microscope mirror. The chief advantages are that the illuminant becomes of uniform intensity and is circular in shape. Its effective size can easily be varied if desired, and, most important of all, its intensity can be altered within wide limits without any change in quality. It is doubtful if this type of illuminant would have been brought into use again had it not been for the invention of the half-watt gas-filled lamp, with its high intensity, and with a spiral filament short in effective length. The effect of this is that a considerable quantity of light can be collected by the speculum, so much, in fact, that even with high powers the intensity is uncomfortably great for visual work. The coiled filament is shown in Fig. 50 *a* magnified by 20 diameters, and from this it will be realised that a considerable portion of the total light emitted can be collected by a glass rod with a diameter of about ½ inch. A further recent development has been the production of what are known as 'coiled-coil' filaments (Fig. 50 *b*) which are still better for this purpose, and the use of this type of clear-glass lamp is advised. The design of the casing of the lamp, which is its chief characteristic, will be seen in Figs. 51, 52, and 53. The base consists



a



b

FIG. 50.

of a square cast-iron frame, A, which supports another similar frame, B, of smaller size. The latter is supported in the former by means of a pivot, C, and clamping screw, D, on opposite sides of the rectangular base, so that the frame B can, within limits, be inclined to any desired angle in relation to the microscope. The light-excluding device consists of two strips of thin metal, such as sheet aluminium, bent to shape as in E and F. Each of these strips is supported on the iron frame B on opposite sides, so that there is a space between them, and they are not in

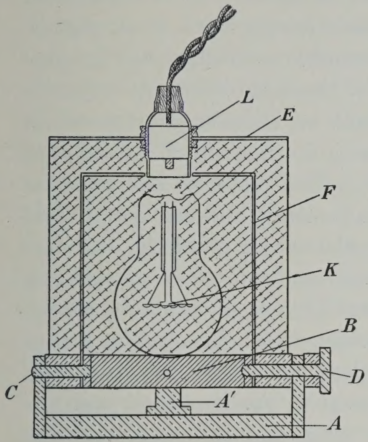


Fig. 51.

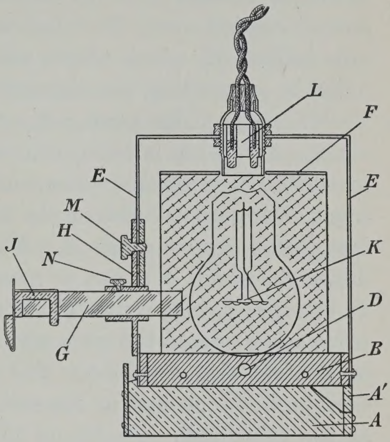


Fig. 52.

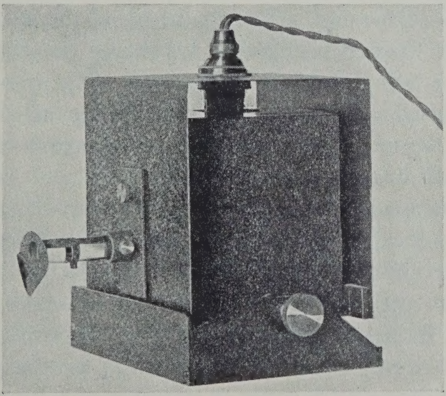


Fig. 53.

contact at any place. The drawings show this effectively. The lamp is supported by the outer casing, but passes through a hole in the inner casing. The result is that little heat is conducted to the outer casing ; it is not too hot to handle even after several hours constant use. The glass rod G is supported in an adjustable holder, H, which allows movement vertically, and is provided to ensure that the filament of the lamp is exactly opposite to the centre of the glass rod. Such an adjustment is essential when a new lamp is taken into use ; the filament is not always in the same position in the bulb. The plate H, therefore, is free to move up or down on the lamp casing, and can be clamped into position by the screw M. The glass rod G is held in a tubular fitting, in which it is free to move to or from the lamp and can be clamped by the screw N. A small fitting, J, is shown on the microscope end of the glass rod which will hold a colour screen or neutral glass modifier. The glass rod should be free from defects, finely ground at the end near to the lamp and polished at the other end. To set up the lamp and to ensure that the rod is opposite the filament, a pin-hole camera is provided. This is a piece of brass tube, of the same size as the glass rod, which can be put in place of the latter. One end of the tube is closed except for a central pinhole, and the other end is open to allow a small piece of ground-glass or translucent paper to be placed over the opening. Thus an image of the lamp filament is thrown on to the ground-glass or paper and the glass-rod holder is adjusted until the image is central. The pin-hole camera is then replaced by the glass rod. The only other adjustment necessary is to move the glass rod either nearer to or farther from the bulb until any desired intensity is obtained, and this adjustment can easily be made to suit any conditions that arise. Such a lamp does provide a simple and inexpensive light-source suitable for taking photo-micrographs with medium and low powers, even with high powers the exposure is not unduly prolonged. With low powers, often the most difficult of photo-micrographic work, it works well, as the light-source is effectively large enough to cover a considerable area. If colour screens are used they should be of moderate density. In most cases the best result will be obtained by placing the polished end of the glass rod a few inches from the sub-stage condenser of the microscope without any intervening collecting-lens.

Another type of electric lamp available is similar in principle to the small bulbs used in motor-car head lamps. These operate on an electric supply of from six to twelve volts, the amperage being high to provide sufficient energy. It is possible therefore to run them from storage batteries, from an ordinary electric supply with suitable resistance in series, or on alternating current through a transformer. The filament is short as a result of the small potential on the terminals of the lamp and the light-source is therefore concentrated, but most of the disadvantages of ordinary filament lamps are still present. Because of the small size of the bulb it is possible to use such lamps in close proximity to the microscope ; in some cases the lamp can replace the microscope mirror. Owing to the thickness of the filament the lamp may be slightly overrun, that is a rather higher voltage may be employed than that marked on the bulb. This results in higher efficiency but tends to reduce the life of the lamp. For many purposes such electric lamps may be recommended, they certainly do provide an illuminant which is simple to use and of fairly high efficiency. A further development is the introduction of 'ribbon filament' lamps in which the incandescent coiled filament is replaced by a tungsten ribbon. Instead therefore of an incandescent wire the illuminant consists of a heated ribbon which increases the breadth of the light-source. Such appliances are to be obtained from all opticians, who also supply transformers for use on the ordinary electric supply to reduce the voltage to that required for any particular lamp. It is important to recognise that the current consumption is considerable and may reach as much as 20 amperes with some ribbon lamps. Secondary batteries therefore must be of large capacity to maintain a constant current output. Transformers must be purchased to suit the lamp to be used, whilst resistances must be wound with wire capable of carrying a large current without overheating.

Whether used with direct or alternating current the electric arc is unrivalled for micro-projection and for this reason its use in photo-micrography has many advantages. All photomicrographs are records of projected images on the perfection of which the quality of the resulting photograph depends. With an arc it is possible to obtain with any objective a projected image that can be seen while the necessary adjustments of the microscope are carried out. Colour screens of almost any visible colour and depth can be tried and their effect observed. The cost of running an arc is not higher than that of most ordinary domestic heaters and carbon renewal is not a serious item. It is often possible to buy second-hand arc lamps, which may be of an old pattern, but which are quite serviceable, providing that their adjustments are still in good condition. For these reasons an example of what might be regarded as an obsolete type of arc-lamp has been included here. It still embodies the required movements and the principles are the same as in some more modern productions.

The difficulty inherent in all illuminants, particularly electrical ones of small area and irregular shape, is to arrange them so that the image of the source of light will cover the object. With metallic filament electric lamps, the filament is of considerable length, but is very narrow; the consequence is that if a direct image of the source of light is projected it is impossible to fill the aperture of any objective. A common way of overcoming this is to enclose the source of light in a ground-glass globe, or to interpose between it and the sub-stage condenser a piece of ground-glass. The objection to this method is that the image of the irregular surface of the ground-glass is projected into the field of view, and produces an appearance which for visual work is disconcerting, and for photographic purposes is absolutely impossible.

Sunlight.—Direct sunlight may be used for some classes of work, but with it some form of heliostat is advisable. It is necessary for this to be adjusted for the particular latitude in which the work is being carried out. In this country it can hardly be regarded as a satisfactory method of illumination and will probably rarely be used. It introduces considerable difficulty in estimating exposures owing to the variability of the actinic value of sunlight. It must be admitted, however, that in the early days of photo-micrography it was necessary, and good results were obtained; but the photographic plates then available were not to be compared in sensitiveness to those of to-day and the optical appliances were relatively crude. The lenses were usually of small aperture, not so well polished, and made of glass of less transparency than at present.

A heliostat designed by Dr. G. Johnston Stoney, which is particularly suited to the requirements of the physicist and photo-micrographer, is made by Messrs. W. Watson & Sons of High Holborn.

A possible substitute for the heliostat where its cost is prohibitive, or where for other reasons it is unobtainable, is the arrangement suggested in the diagram, Fig. 54. Sunlight is allowed to enter the room in which the work is being done in the direction as shown by the dotted lines S. The rays will, of course, be parallel. They strike either on a mirror, which should have a silvered surface, or preferably on a reflecting prism A, and are thence reflected in the direction E, the latter representing the sub-stage optical system of the microscope. If a small subsidiary mirror B be fastened on one edge of the prism, roughly in the position as shown but in such a way that it does not materially cut off the incident ray, then another narrow beam of light is reflected in the direction D. It is evident that the small mirror is so placed that it does not reflect a beam at right angles to the incident beam. The reflecting system should be supported on any suitable stand, and be capable of adjustment about an axis situated at the centre of the reflecting surface of the prism. This movement should be

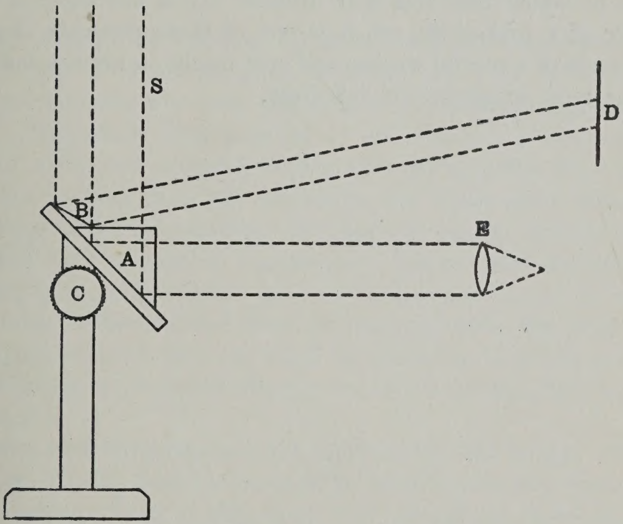


FIG. 54.—Simple Form of Heliostat.

controlled by a large milled head C, or the prism should be supported on a ball-and-socket joint with universal motion. It will be found that if the subsidiary reflected ray is thrown on to any white surface, such as the ceiling or the wall of a room, and its position indicated by reference cross-lines when the main beam of light is in the optical axis of the microscope, it is possible by moving the prism whilst watching the spot D, to keep the rays of light truly in the axis of the microscope.

Some similar arrangement has been used for photographing stars, and the writers have successfully worked with an arc lamp in which this idea was utilised. It is obviously of the nature of a makeshift, yet it is one of those methods that in the hands of a careful worker will give results in no way inferior to the most expensive of heliostats.

CHAPTER VI

THE PHOTO-MICROGRAPHIC CAMERA

Simple Forms of Vertical Camera.—Having considered the optical part of the apparatus, reference must now be made to that portion which is more particularly of a photographic character. Essentially this consists of an ordinary photographic camera, and it will probably happen that the majority of workers who are starting from the beginning will make some attempt to obtain photo-micrographs with such a camera used in conjunction with a suitable microscope. Under these conditions, all that is required is that the microscope shall be set on some firm table or bench, and that the camera, with the ordinary photographic lens removed, shall be placed so that the microscopic image is projected directly on to the ground-glass focussing-screen.

Under such circumstances the only points that require attention are, firstly, that the camera be placed with the centre of the focussing-screen in alignment with the optical centre of the microscope, and, secondly, that the focussing-screen be exactly at right angles to the optical axis.

It is evident that such an arrangement is open to considerable objections, since, although it is quite possible to obtain good photographs when using low powers, it becomes a matter of considerable difficulty if the magnification exceeds some twenty or thirty diameters; for there is no arrangement to ensure that the relative position of the microscope and camera is unaltered during the process of focussing and exposing.

Nearly all who take up this work make an effort at some time to build an apparatus according to their own ideas. Such an arrangement, in the hands of an intelligent worker, often gives the best results. Each one will have ideas which as the result of experiment they would like to carry into practice, and an apparatus built up as the result of practical experience is usually most satisfactory.

The designs for an apparatus may be divided very broadly into two well-defined types. In one arrangement, and probably the one which has the most to recommend it, the microscope and camera are supported on one very stiff and solid base, so that no alteration of either part in relation to the other can inadvertently take place. In the other type the microscope and camera are treated as two separate pieces of apparatus, the idea in this design being that where any outside influence tends to disturb the position of either of the components, it would only affect one of them. For instance, if in the course of focussing the image on the screen of the camera, in inserting the dark-slide or in withdrawing the shutter, some alteration takes place in the position of the camera, the microscope and its adjustments are not affected. It is claimed for this method that the greatest evil would result from any movement of the microscope—and such is unquestionably the case—therefore that the microscope should as far as possible be isolated and be free from such disturbing influences. One of the greatest troubles met with where the work is carried out in an ordinary room or laboratory is vibration or tremor. Well-defined shaking of the apparatus is rarely a factor, as any worker would usually take reasonable precautions against dislocation of the apparatus, but it is the ordinary tremors that are most difficult to provide against. It would appear that the type of apparatus in which everything is supported on, and, if thought advisable, clamped down to, a solid and heavy base, would be the better type ; in such case any vibration affecting the apparatus would affect the whole of it equally, and no disturbance of the components in relation to each other would result.

Where a simple type of apparatus is decided on, and where no special bench or supporting-table can be devoted to the work, it is probable that a vertical camera is the best type. In this the microscope is kept in a vertical position and the light therefore has to be reflected by the mirror through the substage condenser, as under ordinary conditions in visual work. The camera is supported over the microscope so that the image is thrown on to the focussing-screen, which therefore lies in a horizontal position. This type of apparatus has limitations; the length of camera can hardly exceed 24 inches, and consequently, unless high-power projection oculars are used, high magnifications are not obtained.

For the purpose, however, of recording the results of researches, this apparatus is extremely useful. One of the simplest forms is that shown in Fig. 55, made by Messrs. J. Swift & Son, and all other opticians. It consists simply of an aluminium cone which fits over the upper end of the body-tube of the microscope. It is not more than about 12 inches in length, and on its upper end carries the ground-glass focussing-screen or the plate-holder. For ordinary laboratory work it is a very useful piece of apparatus; but its use requires care, owing to the fact that in inserting the dark-slide, or in withdrawing the shutter of the latter, there is considerable danger of altering the focus of the microscope, the camera in effect being a part of the microscope.

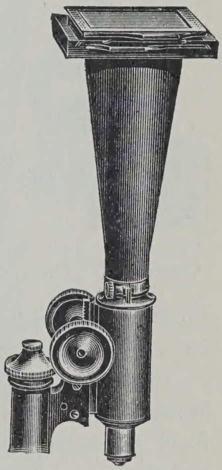


Fig. 55.—Simple Form of Photo-micrographic Camera. (James Swift & Son, Ltd.)

A type of vertical camera which admits of rather wider application is made by Zeiss, Leitz, and others, in which the camera is supported separately from the microscope (Fig. 56). A stiff steel rod is supported on a tripod foot; the camera, both at the lower end near the microscope and also at the frame which carries the focussing-screen, is fixed to the rod by clamps and is capable of adjustment in a vertical direction. The microscope stands on the table, so that there is no rigid connection between it and the camera. This type is convenient, since the camera can be bodily removed, or can be swung round on the supporting-rod when observations have to be made, and replaced over the microscope at will. It is, however, open to the objection that the support of the focussing-screen or the dark-slide is at the end farthest away from the tripod, and thus we have much the same conditions as obtain when a metal bar is rigidly sup-

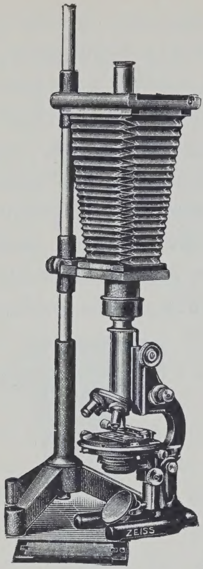


Fig. 56. — Vertical Camera. (C. Zeiss.)

ported at one end only—namely, that the rod is specially subject to any vibration. Such vibration would result in the greatest movement of the upper end of the camera, and consequently would have the worst effect on the definition of the photographs.

A further development of the same type is that in which the camera is still carried on a vertical rod, but the base is so made that the microscope can be placed on it. There is also an arrangement by which the camera can be used in either a vertical or a horizontal position (Fig. 57); further, the camera is so supported that when used in either position it can be swung out of the way for the purpose of making any observation. Although this type has a firm base, carrying both camera and microscope, and the component parts of the apparatus form one unit, it is open to the same objections as the previous one, the camera itself is liable to vibration owing to its method of support. While it is a useful form of apparatus for ordinary laboratory work, it should not be used in preference to a stable horizontal form, since it is designed chiefly for obtaining photographic records of researches.

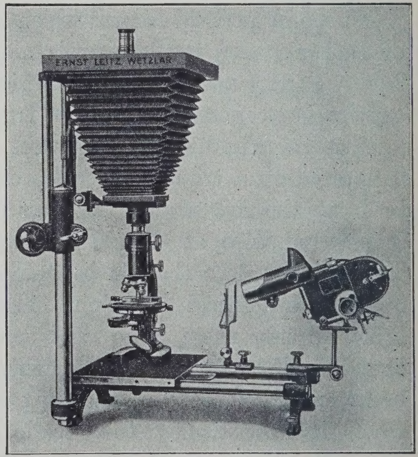


Fig. 57.—Combined Horizontal and Vertical Camera. (E. Leitz.)

Some form of vertical apparatus is sometimes, however, a necessity, and the most satisfactory is that known as Van Heurck's vertical camera, made by Messrs. W. Watson & Sons of High Holborn

(Fig. 58). This apparatus is particularly rigid, and, as the illustration shows, consists of an elongated box supported on either side by strong supports, and carrying the focussing-screen and dark-slide at its upper end. On the side of the box near to the observer is a door which, while it can be opened for making any adjustment to the microscope, is light-tight when closed and ready for taking a photograph. The chief objection is that there is no method of altering the distance between the microscope and the focussing-screen, so that any variation of magnification has to be effected by a suitable combination of objective and ocular. The apparatus is very convenient for photographing objects that lie in fluid, or in cases where the microscope must remain vertical.

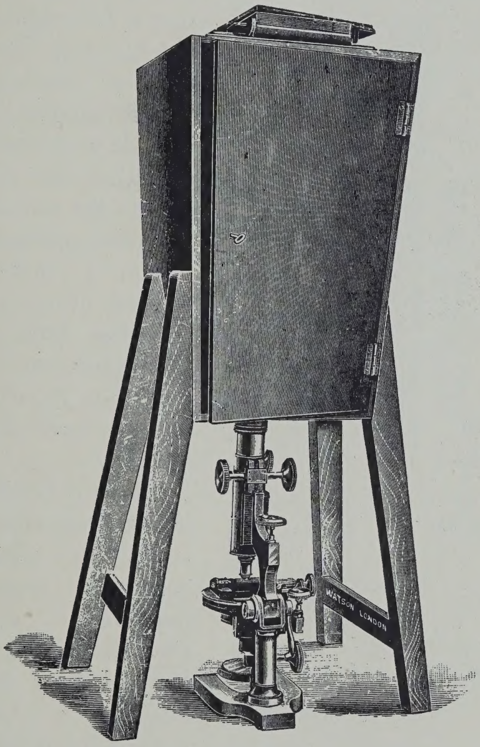


Fig. 58.—Van Heurck's Vertical Camera. (W. Watson & Sons.)

Modern dark-ground illumination methods bring the photography of objects such as living bacteria within the bounds of ordinary work, and the Van Heurck apparatus is eminently suited for this purpose. The table on which the microscope is supported should be strong and stiff, and it is essential that the microscope and camera stand on a level surface. The apparatus as shown is supported on four points, and mechanically this is unsound. The writers' modified apparatus is made with one strong leg on either side, and a third one on the side opposite to the observation-door. The worker is therefore independent of any inequalities of either table or bench; the apparatus being supported on three points will in any case stand quite firmly.

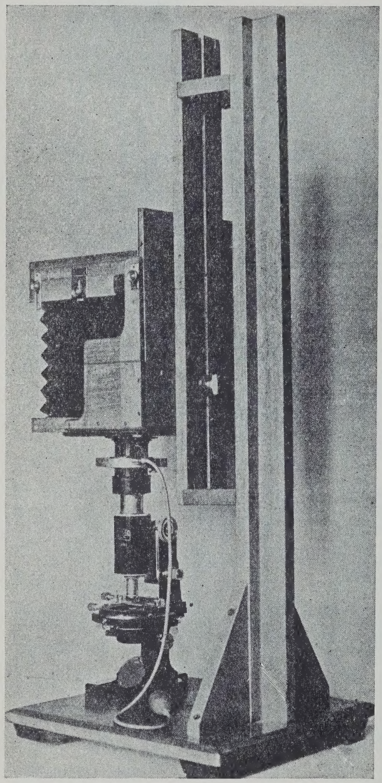


Fig. 59.—Ordinary Camera used as Vertical Camera.

Where a vertical camera is required simply as an adjunct to a more complete horizontal apparatus an improvised arrangement such as Fig. 59 is as good as many at present on the market. Anyone with a slight knowledge of carpentry can make it, or it can be constructed at small cost by a mechanic. It consists simply of a baseboard about twelve to fourteen inches square, and one inch thick; underneath this should be screwed two cross bracing pieces of the same thickness in a direction across the grain of the wood, so that the chance of warping is reduced to a minimum. Two other lengths of wood of the same thickness, each about three inches broad, should be fastened together in T-form, as shown in the illustration, and these should be supported at right angles to the baseboard and firmly screwed to it. On the flat side of this vertical piece, another thinner piece of wood should be placed in which is a narrow slot about three-eighths of an inch broad; any long-extension photographic camera can then be clamped to this with a suitable clamping-screw, and be raised or lowered into any required position. The microscope is placed below the camera, the image being projected upwards on to the focussing-screen. Such an arrangement allows large objects to be photographed

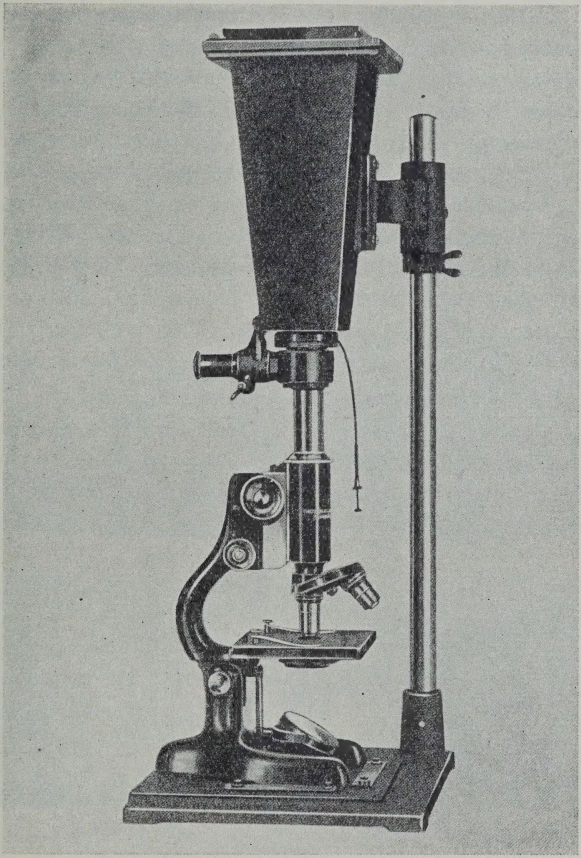


FIG. 60.

that do not require the use of a microscope, and yet that readily come within the purview of a photo-micrographer. Many objects, such as bacteriological tubes and plate-cultures, may be dealt with more conveniently if they are lying flat than when supported in front of a horizontal camera.

One of the objections to any vertical appliance is the necessity of observing from above when focussing the image on the ground-glass screen. To do this in comfort the apparatus must be mounted on a low table and this is not convenient for ordinary microscope work. To obviate this difficulty a reflecting type of ocular can be mounted on the body-tube of the microscope. A camera with this attachment to the microscope, made by Messrs. Bausch and Lomb, is seen in Fig. 60. The image is focussed in the side tube, which is provided with adjustments to ensure that when the image is so focussed it is also in correct adjustment on the focussing-screen. This addition results in considerable increase in cost, but it is a great convenience.

Horizontal Cameras.—Of the horizontal types of apparatus, Fig. 61 is an illustration of the simplest form. A plano-convex collecting-lens, microscope, and camera, are placed on a wood base in their correct relative positions; no arrangement is made for ensuring that they are in alignment or for enabling them to be replaced should either of the parts be removed. Such an apparatus would usually be improvised by the worker out of an ordinary long-extension photographic camera. It may, however, be improved with little trouble and at small cost. Guides for the microscope should be placed on the baseboard, so that it could always be replaced in its proper position, and the same might be done with the lamp and the bull's-eye condenser.

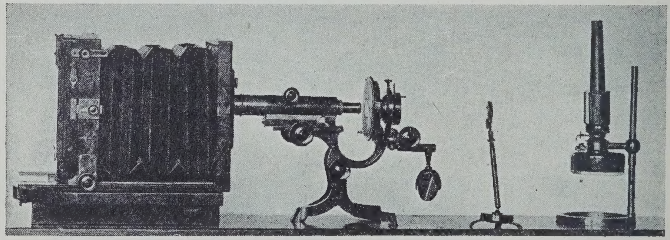


FIG. 61.—Simple form of Horizontal Camera.

The camera has to be kept very short, so that a worker while observing the image on the ground-glass focussing-screen can reach the fine- or coarse-adjustment of the microscope. The possibilities of the apparatus, therefore, are limited, and the work which it is capable of doing would be confined to the use of low-power lenses.

The next step towards greater elaboration should consist of an elongation of the baseboard, making it possible to use a camera with greater bellows extension. When this is done it becomes necessary to have an arrangement for controlling the fine-adjustment of the microscope while the observer is watching the image on the focussing-screen. There are many ways of doing this, and a simple one is that shown in the illustration (Fig. 62). A long metal rod is supported on the wooden base and has a large milled head at its end near the focussing-screen. At the other end is a grooved pulley, and between this and the fine-adjustment of the microscope a connection is made, usually consisting of a light endless cord which passes over both. This is preferably a silk thread well rubbed with bees'-wax so that it is not liable to slip on either pulley; in any case it should be taken from one to the other so that there is no strain. It will be found that with waxed silk a loose connection will turn the fine-adjustment screw, and that it will respond immediately to changes of direction. Some inexpensive additions to this type of apparatus will increase its efficiency. It is convenient to be able to make an observation in the microscope, or to alter any of the adjustments while observing the image directly through the instrument. This may be effected in two ways: either by carrying the microscope and the illu-

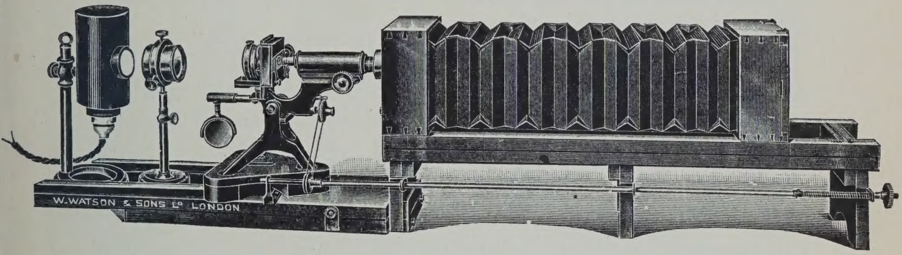


FIG. 62.

minant on a separate board or support, as shown, and so arranging that it can be swung out on a central pivot as an axis; or, as in the types of apparatus devised by Messrs. Zeiss, the microscope itself may be on a sliding base so that it can be moved laterally. The former method is preferable, as no alteration results in the relative position of the optical system and the illuminant: when the turntable is swung it allows an observer to adjust the light, or any of the illuminating apparatus, without having to watch the effect of any such alteration on the focussing-screen of the camera.

The apparatus (Fig. 62) embodies both the arrangement for focussing from a distance, and also a simple form of turntable carrying the microscope, and, as it is stiffly built, it would answer for high-power work. If, however, an apparatus is required suitable for any class of work, then still further elaboration is advisable.

The apparatus used by the authors in the department of Applied Optics, National Institute for Medical Research, is seen in Plate IV, Figs. 1 and 2. It cannot be too strongly emphasised that elaboration of apparatus need not result in better photo-micrographs; in fact, as with many other technical processes, elaboration may actually be a hindrance. This has been kept carefully in view in building up the appliance described, it is simple in its essential design and easily adaptable for any branch of work; but so far as its optical parts are concerned the only question considered has been suitability, the products of any optician having been used without regard to their origin. The principle is that the illuminant, microscope and all subsidiary appliances are mounted on an optical bench of triangular section, and the whole of this can be swung out on an axis situated near the centre of the arc-lamp shown. The camera is mounted on a geometric slide, as described later in this book, this giving great stability as the points of support are widely separated. To facilitate adjustment a small white screen (Plate IV, Fig. 3) is supported at the side of the camera in alignment with the focussing-screen, and swings on a vertical axis so that it can be set at right angles to the direction of the optical bench when this is swung out. The components, as shown, are any good arc-lamp or other illuminant in a casing which prevents light from escaping except in the direction of the microscope;

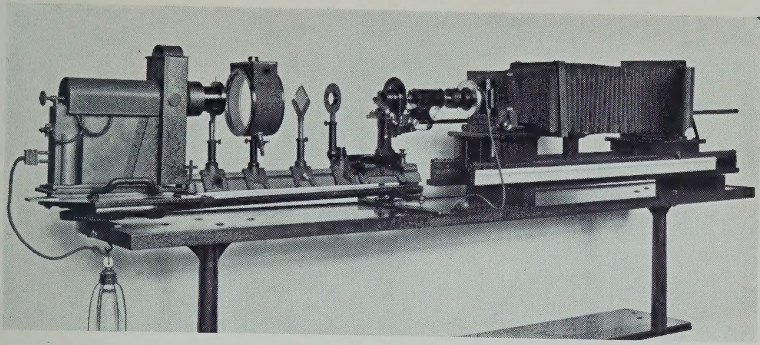


FIG. 1

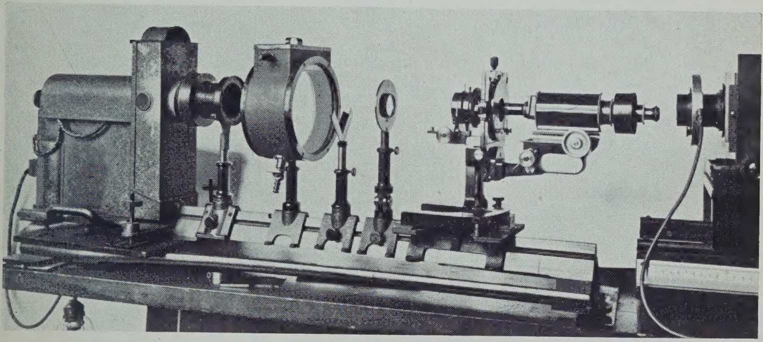


FIG. 2

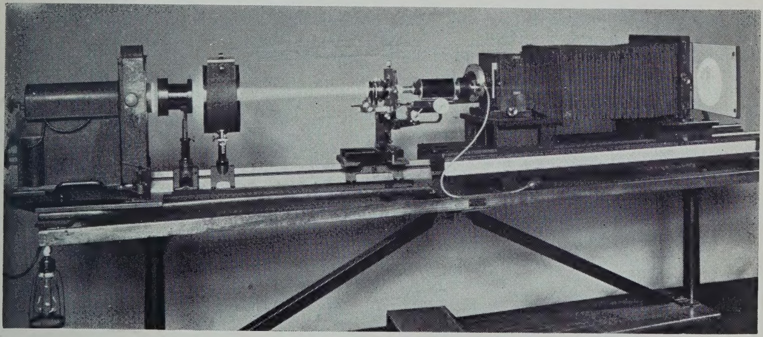


FIG. 3

Authors' Photo-micrographic Apparatus.

a collecting-lens, which is of the type described on page 83, but for which any other suitable lens may be substituted. The water-bath and screen-holder are placed in the order shown, and a long-focus condensing lens for low-power work is used if required. The microscope is on the end of the bench and is clamped to its saddle. The camera is in two sections, so that it can be used as short as six inches or lengthened to as much as four feet. The optical bench is removable so that any ordinary photographic lens can be placed on the camera front and more than half the supporting-table is available for suitably arranging on it any large object which it is desired to photograph. The method of actuating the fine-adjustment from the focussing-screen end of the camera is by means of a long wooden rod, and the connection to the microscope is by means of Hooke's joints substantially in the form made by Messrs. Zeiss. Although there is slight loss of time it is not sufficient to prevent very accurate focussing, as the movement stops with precision and this is the point that matters. A scale is provided to indicate the camera length from the ocular front, a useful adjunct for approximate estimation of magnification. A simple form of photographic shutter is provided, but is unnecessary unless very short exposures are needed.

Plate IV Fig 1 shows the whole apparatus on its stand with the parts described in position. The disposition of the constituents from left to right is as follows :—

Automatic Arc-lamp.
Aspherical Condensing Lens.
Water-bath.
Colour-screen in Holder.
Spectacle Lens for Low-power Work.
Microscope.
Camera.
End of Focussing-rod.

Plate IV Fig. 2. The former parts shown in greater detail. The commencement of the camera-length scale can be seen.

Plate IV Fig. 3. The apparatus is here shown in action with the illuminating beam and projected image. It will be seen that the beam when it leaves the condensing-lens is convergent so that an image of the light-source is focussed at the position occupied by the iris-diaphragm in the sub-stage. The spectacle-lens has been removed. The additional struts shown under the table have recently been added to overcome sagging of the table-top, a point to bear in mind in constructing any such apparatus when considerable weight is carried on a long length.

A more complete apparatus and one embodying arrangements which differ somewhat from those already described, is the photo-micrographic camera of Messrs. Zeiss. This is of the second type already mentioned in which the table carrying the optical parts is separated from the camera and its support; there are, therefore, two separate pieces of apparatus. The microscope is carried on an adjustable metal plate, which allows of its sliding at right angles to the optical axis, hence it can be moved out of position for any adjustment to be made without interfering with the remainder of the apparatus. It consists of two tables, one carrying the camera and the other a triangular bar upon which all the optical parts as well as the illuminant can be fixed, although in later forms the latter is supported on the table at the end of the triangular bar. All the optical apparatus is carried on saddles which may be adjusted and clamped in any position on the triangular bar. The sole-plate carrying the microscope has levelling screws and elevating gear, so that any microscope of less height than that for which the apparatus is designed may be used on it. The method of focussing from a distance—that is, from the position of the focussing-screen of the camera—is by means of rigid rods of wood or metal which are connected together by means of flexible joints (Fig. 63); there are no cords or similar flexible connections as in the apparatus previously described. A bell-shaped metal cap slips over the fine-adjustment screw and can be clamped on to it. An arm in connection with this clamp goes to a column adjustable for height, which is fixed on the table at the side of the triangular bar; by means of a Hooke's joint a flexible yet rigid connection is made between the focussing-rod which goes to the camera, and the rod connected with the fine- or coarse-adjustment of the microscope.

The illuminant supplied with the apparatus consists of an automatic electric-arc lamp, a description of which is embodied in the chapter on Illuminants. The illuminant is not carried on the metal bar, but is simply placed on the table at the end of it; the collecting-lenses, as well as a water-cooling chamber, are, however, carried on the bar. The camera-base, on its support, is constructed substantially as shown in Fig. 64, and is so designed that either of the component parts are adjustable along the metal supporting rods, and can be clamped in any position; or the whole can be moved to and fro so that it is easy to get at the microscope and to make any adjustment. In later forms of the apparatus the displacement of the entire camera is arranged by sliding it on two rollers, so that it can be quickly and easily adjusted. By means of milled heads at the side it can be clamped when the necessary adjustments have been made. The camera is stiffly supported so far as its base is concerned, but is liable to feel the effect of any tremor imparted to it. At the same time, some of the finest work has been done with this apparatus, but it is essential that the position in which it is set up is free from any influence likely to cause relative movement of any of the parts.

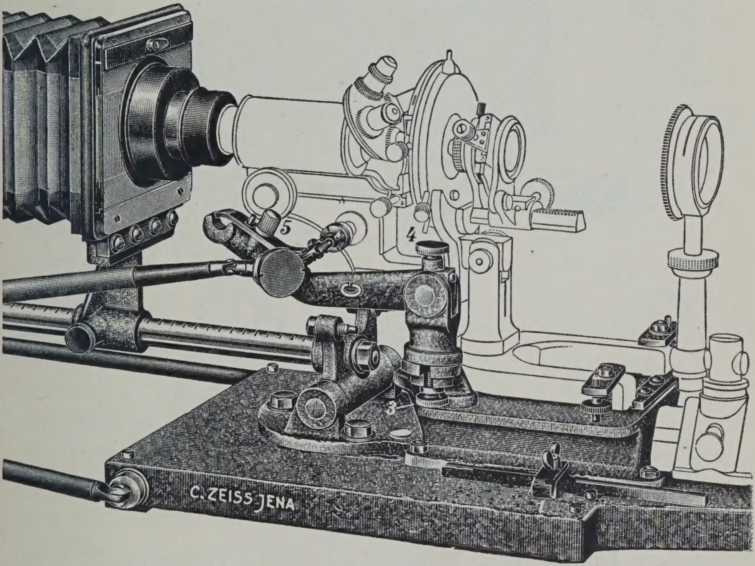


Fig. 63.—Method of Controlling Fine-adjustment. (C. Zeiss.)

Cameras of various types are also made by Messrs. W. Watson & Sons, Charles Baker, R. &. J. Beck, and J. Swift & Son; but as none of these embody points that are entirely novel, a detailed description of them would not appear to be necessary; reference should in each case be made to the makers' catalogues.

The writers cannot too strongly recommend anyone who may contemplate building up an apparatus to study the principles of the geometric slide as enunciated by Lord Kelvin.[1] Although it is admitted that for heavy machines it might have disadvantages, it far surpasses any other method for the purpose now being discussed. The accuracy of any apparatus so built is far less dependent on perfect workmanship than in any other design.

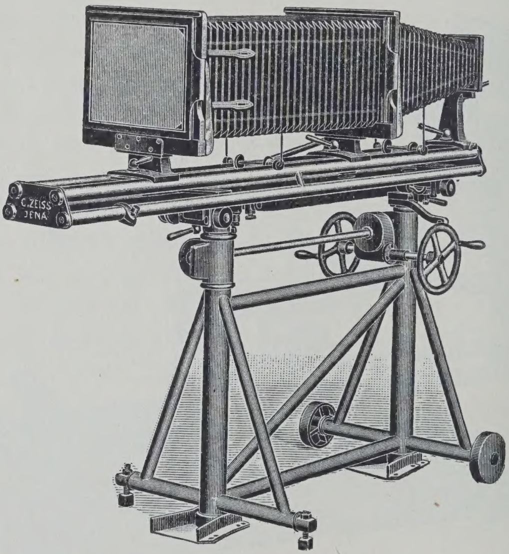


Fig. 64.—Large Micrographic Camera. (C. Zeiss.)

Fig. 65 illustrates the geometric slide and the method of construction. Let B represent the baseboard of the proposed camera, on which are fixed two metal rods or tubes A, A (these should preferably be brass-cased iron tubes of about three-quarters of an inch in diameter, as they are stiffer and cheaper to purchase than rod or tube of solid brass). Any part of the apparatus to slide along these rods should be supported on a piece of wood which has two V-shaped grooves (C, Fig. 65) in line along one side of it, and a plane surface on the other side, D. The two V-grooves should be as far apart as may be convenient, so that a longer support is given to the apparatus; but in the case of the focussing-screen of the camera, or any similar part, a distance from end to end of five or six inches is ample. When any part of the apparatus sliding in this manner is to be clamped down, one screw only is necessary, and this should pass through the sliding-plate at the centre E of a triangle, which is formed by the ends of the two V-groove slides and the centre of the plane point of support on the other side as shown at F. No part of this arrangement requires to be made with any particular accuracy. The two faces of the V-grooves are preferably at right angles to one another, so that when clamped down there is no tendency to jam, and also to ensure that the movement is sufficiently free when the clamping-screw is released.

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[1] Thomson and Tate, *Treatise on Natural Philosophy*, Part I, p. 154.

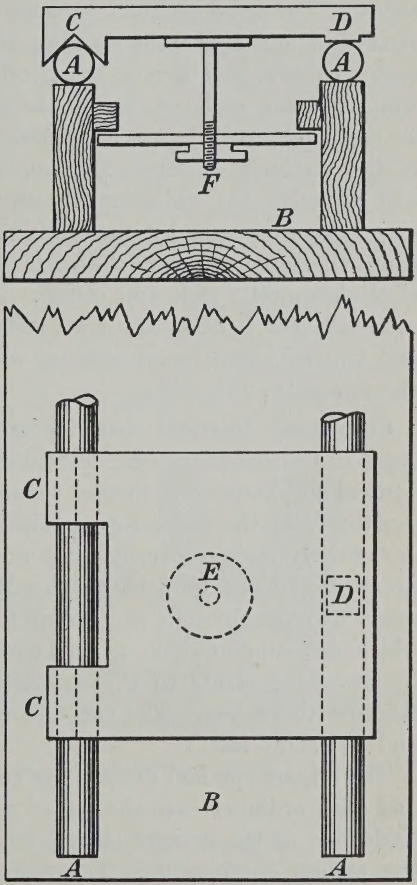


FIG. 65.—The Geometric Slide.

In placing any piece of apparatus on such a geometric slide advantage should be taken of the method—also set out by Lord Kelvin—of three points of support (see Fig. 47). If any piece of apparatus resting on three points is set horizontally it then has freedom to rotate on a vertical axis only, and when clamped down has no power of motion perpendicularly to that plane. The ends of the three points of support should be rounded, and one of these should be dropped into a shallow conical hole; the apparatus then has freedom of rotation only about this one point. The second point should then be placed in a V-groove which runs in a direction as shown. The third point of support is on a plane surface. It is clear that any apparatus standing in this manner has no freedom to move, and if it is taken off its base for any purpose, will, on being replaced, be absolutely in the same position it occupied originally. It is very difficult to claim this for many of the methods of supporting apparatus now in general use, and although the objections to their design may to a large extent be overcome by good workmanship, they would never reach the same accuracy that can be obtained by this method of the geometric slide and clamp. In a simple form of apparatus all that is necessary is a baseboard to support the whole, and two long stiff metal rods on which all the components of the apparatus can slide.

Combined Vertical and Horizontal Cameras.—Various types of camera have been devised embodying the two designs vertical and horizontal, so that the camera may be used in conjunction with the microscope in either position. None of them are entirely satisfactory, as they are of the nature of a compromise. In one brought out by Messrs. Zeiss an attempt is made to overcome the great objection to all vertical cameras which are supported on a single metal bar. This is effected by attaching struts to the supporting-rod so that it has no freedom to vibrate. The accompanying illustrations (Figs. 66 and 67) show this.

The microscope and camera are supported on one solid base, and with ordinary care the act of removing the shutter in the dark-slide of the camera should not result in displacement of the various components. The base consists of a heavy cast-iron plate separated into two parts, the front portion supporting the microscope, and the rear part serving as a support for the camera. The focussing-screen of the camera as well as the front is adjustable, and can be set in any position on the supporting-rod. By means of a groove in this rod the parts are prevented from rotating, having freedom of movement in a vertical direction only. The bellows are fixed by a hinge and catch to the frame carrying the focussing-screen, so that if the catch be loosened, the bellows drop down at one side, and the image of the object may be viewed on a dead-white reflecting-screen. Vibration of the supporting-rod is prevented by having two telescopic struts supported at their lower end from the corners of the base-plate, and can be loosened when any alteration of camera-length is necessary. They may be clamped at any position when the desired length of camera is obtained.

When in the vertical position the camera is also available for photographing large objects, any suitable lens being screwed on to the front of the camera in the ordinary way. An additional camera-front is supplied for carrying the flanges of any such lenses.

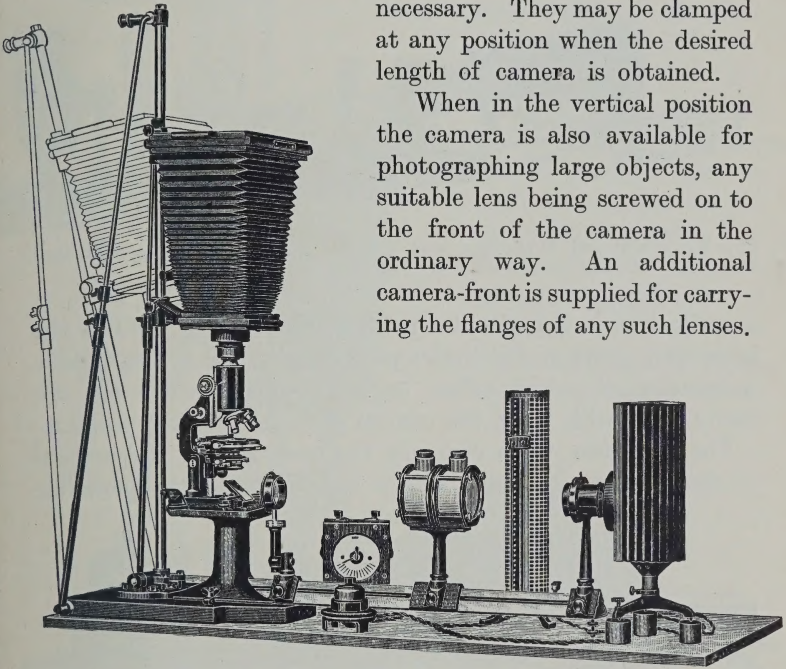


Fig. 66.—Combined Horizontal and Vertical Camera, about $\frac{1}{15}$ full size (C. Zeiss.)

Two focussing-screens are supplied, one ground-glass and the other plain glass, as with other cameras. The base-plate supporting the microscope rests on three levelling-screws, and can be clamped down when once the microscope has been adjusted in position. It can also be removed should the camera be required for other purposes. This base-plate, carrying the microscope, can be rotated so that the camera is available on either side of the operator. The illuminant and optical accessories are carried on the optical bench in the same way as in the larger horizontal camera already described. The optical bench has a pin at one end which drops into a hole in the base-plate, so that it always returns to its proper position in relation to the microscope. The other end of the optical bench is supported on the table on which the apparatus rests.

Arrangements are also made for altering the position of the optical bench according to whether the camera is required in a vertical or in a horizontal position. It may be used in the horizontal position for macro-photography, for photographing bacteriological culture-tubes, or large objects which are not easily dealt with when the camera is in the vertical position.

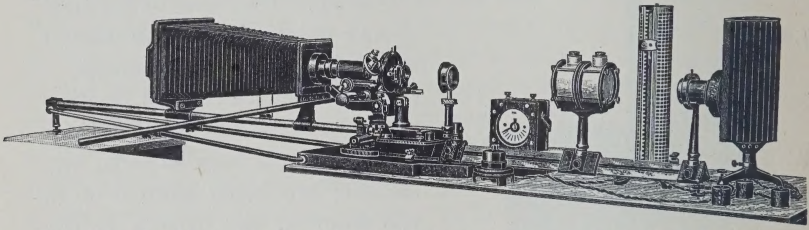


FIG. 67.—Combined Horizontal and Vertical Camera, about $\frac{1}{15}$ full size. (C. Zeiss.)

The procedure when changing the camera from the vertical to the horizontal is quite simple and is effected by loosening the clamps on the vertical rod, and also on the bracing-stays, which at once allows the camera to be tilted over. The accessory apparatus on the optical bench has then to be brought to the proper height so that it is in alignment with the optical centre of the microscope; the adjustments are the same as in other types of horizontal apparatus.

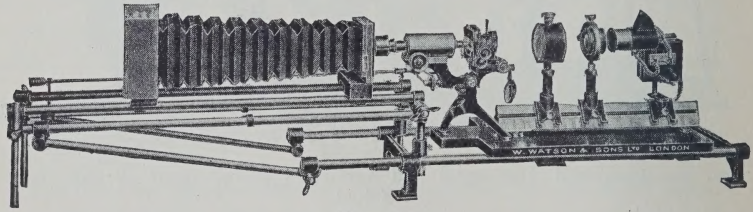

W. WATSON & SONS LTD LONDON

FIG. 68.

A similar apparatus is the 'Duplex' camera made by W. Watson & Sons, Ltd. (Figs. 68 and 69). From the illustrations it will be obvious that the design is the same as that made by Zeiss except that when used with the microscope in the vertical position the illuminant is inclined. In the Zeiss apparatus the microscope is raised so that the illuminant is not altered in position, the whole illuminating system, in fact, remaining horizontal. This has the advantage that no re-adjustment of the illuminating beam is necessary, but it has the disadvantage that it raises the height of the focussing-screen and is not so convenient to get at when focussing. In deciding on the selection of such an appliance it is therefore well to bear these differences in mind, in particular it should be recognised that there is greater difficulty in adjusting any microscope equipment with an inclined illuminating system.

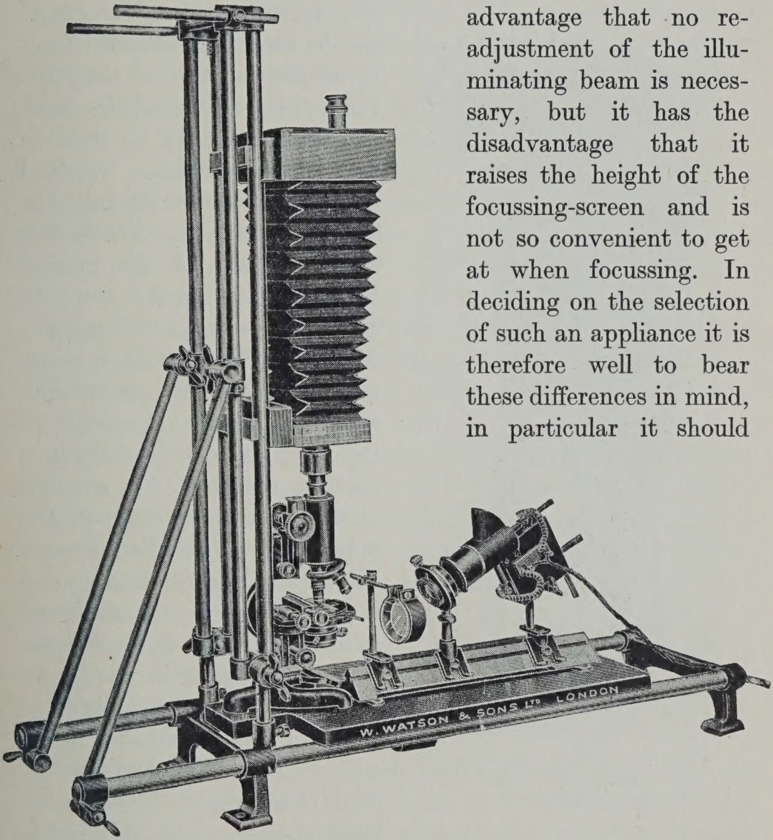


FIG. 69.

With the introduction of improved arrangements for illuminating living bacteria and other unstained objects it is desirable to have an apparatus which is suitable for photographing them. The essential point is that it should be possible to observe the microscopic image, and to focus that image until the desired appearance is seen; then to take an instantaneous photograph at the exact moment when this appearance is observed and the object is in focus. A simple way of doing this is to utilise an ordinary reflex photographic camera (Fig. 70. In these cameras, an object can be seen and focussed right up to the moment of exposure. For photo-micrographic work one of these may be used in conjunction with a vertical camera of the Van Heurck type already described. It is obvious that a camera of the vertical type is more useful for this class of work, as it almost invariably happens that living microscopic objects are in fluid, and it is therefore essential for the microscope to remain in a vertical position. The ground-glass focussing-screen at the upper end of the Van Heurck camera should be removed, and in its place a wooden frame fixed to carry the reflex camera. This frame should have an opening in its centre sufficiently large for any microscopic image to be projected through it. The photographic lens on the reflex camera, and if possible the whole of the front carrying the lens, should be removed. The camera must be turned over in the position as shown, so that it allows the fine-adjustment of the microscope to be reached while observing the image on the ground-glass screen. The only limit to the employment of this method, so far as high powers are concerned, is the intensity of the illuminant used. With any dark-ground-illumination method,

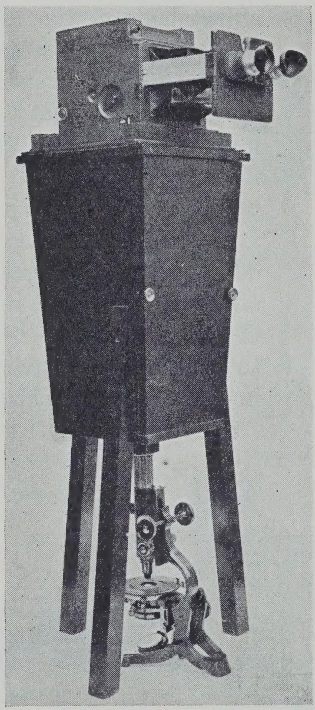


Fig. 70.—Reflex Camera used in combination with Van Heurck Vertical.

the light-source must be powerful to obtain an instantaneous photograph, and it is very doubtful whether anything less than an electric arc would be of service. The reflex camera utilised for this purpose, although it may be of simple design, should be such that it does not cause any appreciable amount of vibration on releasing the reflecting mirror and effecting the exposure. Also the period elapsing between the release of the mirror and the actual exposure of the plate should be short, so that if any vibration is started, it will not be transmitted to the rest of the apparatus before the exposure is completed.

Perhaps the most interesting advance in photo-micrographic method that has taken place is the provision by both Carl Zeiss and Leitz of small light-weight cameras that can be placed at will on any microscope under ordinary observational conditions. The advantage of this is obvious to research workers who want records of observations as they progress. These camera attachments are so efficient that the necessity for large appliances, occupying considerable space and entailing substantial outlay for accessory apparatus, is in many cases completely done away with. The results need not be inferior to those obtained in any other way, in fact, their simplicity in use facilitates the whole process considerably. There is the further advantage that not only can they be used for photographing permanent preparations, but, provided a suitable light-source is used, records of objects in motion can be obtained. The object can be observed up to the moment of exposure, or if some loss of light is unimportant, observation can be continued while the exposure is made.

The ' Micca ' Micro-camera Attachment, made by Leitz (Fig. 71), provides a simple and practically self-evident means of obtaining photo-micrographs on plates measuring 6 × $4\frac{1}{2}$ cm., and the ' Mikkam ' is a larger form to take plates 9 × 12 cm.

The apparatus can be used without any troublesome interposition of adapters and without any special preparation of the microscope stand. It simply takes the place of the usual eye-piece, and it may be used with any microscope stand having a tube of standard diameter.

The smaller micro-camera attachment, exclusive of dark-slide, weighs only 220 grammes (8 ounces). This small weight renders it unnecessary to provide any special means of locking the motion of the tube. It is not even necessary to counteract the motion of the draw-tube, if the latter is in a normal condition. Where the draw-tube moves too freely it will be sufficient to slip a rubber ring over it.

The attachment is designed for use with periplanatic eye-pieces, and with these furnishes faultless pictures. With every camera a periplanatic eye-piece, × 12, is supplied, which is well adapted for this purpose.

The attachment is fitted at the side with a tube through which the image can be viewed during the actual exposure. The prism of the viewing eye-piece can be put out of action so long as pressure is maintained upon a wire release. By this means the whole of the light can be rendered available for taking instantaneous photographs.

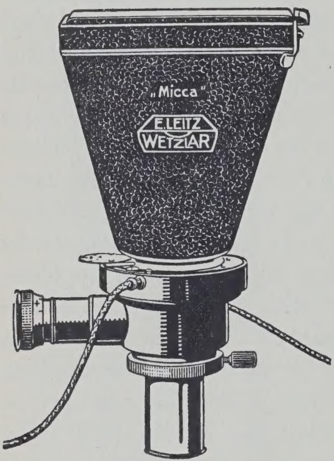


Fig. 71.—The 'Micca' Micro-camera Attachment. (E. Leitz.)

The image seen through the side-tube has the same magnification as that seen directly through the microscope, and with eye-pieces up to the periplanatic eye-piece, × 12, the field of view is likewise the same. The size of the image on a 6 × $4\frac{1}{2}$ cm. plate corresponds to the field of view.

For use the cross lines seen in the side-tube should be focussed sharply by means of the rotating collar. The image should then likewise be sharply focussed by the microscope fine-adjustment while looking through the side focussing-tube, the latter being in exact correspondence with the focal plane of the sensitive plate. The ground-glass plate should in the main be used only for gauging the brightness of the image and for estimating the required time of exposure, since the relatively coarse grain of the ground-glass surface vitiates the observation of fine details.

The shutter is available for time and instantaneous exposures. The dark-slide, when placed in position engages in two grooves, from which it may be released by simultaneously pressing with the thumb and middle finger upon two small levers. This is an essential provision as it is important that no disturbance of the microscope adjustments shall take place when inserting the dark-slide or withdrawing the shutter.

The Siedentopf Photographic Eye-piece (Fig. 72), made by Zeiss, is similar in design. It screws into the outer tube of the larger Zeiss model microscopes and extension collars or adapters in some cases are needed. The attachment increases the tube-length to 230 mm., and to compensate for this achromatic negative lenses are supplied, which also diminish curvature of field and so result in a larger area of perfect focus. Under these conditions the makers recommend the use of apochromatic objectives. The image is focussed in a side-tube as shown in the illustration, the eye-piece being provided with a graticule which must be sharply focussed for each observer, so that reference to the ground-glass screen is unnecessary. To determine the exposure a Goldberg wedge is provided. The only practical point that requires attention, and which is not mentioned in the makers' instructions with these appliances, is to see that no extraneous light reaches the plate through the observing ocular while the exposure is being made. The negatives obtained are small, but subsequent enlargement is practicable, and the ultimate results are not inferior to those obtained by any other method.

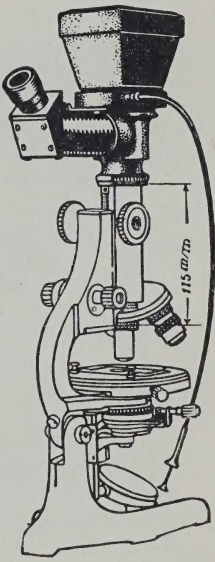


Fig. 72.—Siedentopf Photographic Eye-piece. (C. Zeiss.)

The large photo-micrographic apparatus by Messrs. Leitz of Wetzlar embodies improvements which render it possible to obtain results with great rapidity and certainty (Fig. 73). A full description can be obtained from the makers, so that it is unnecessary to do more than refer to its characteristics. All optical parts as well as the camera are carried on an optical bench of triangular section. The suspension is of new design and is devised to prevent the transference of shock or vibration to any working part. Another unusual feature is that focussing is done by means of a telescopic magnifier, enabling the final image to be observed on a screen. A special microscope is recommended with interchangeable tubes, in one of which an auxiliary eye-piece tube is mounted at right angles to its axis, so that the image may be observed at any time. Improved collecting lenses have been introduced and in general the apparatus is of a most complete character. Where cost is no obstacle it can be recommended as a thoroughly efficient and perfectly constructed apparatus.

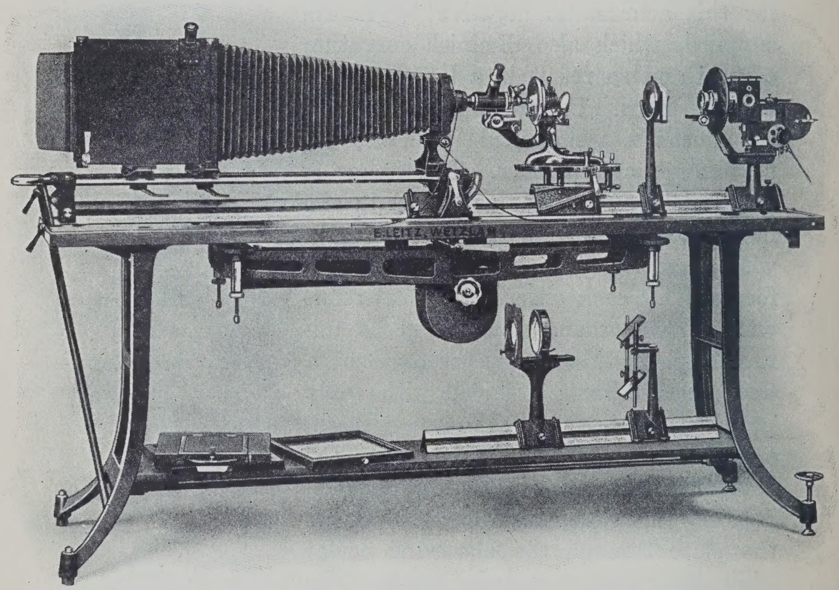


Fig. 73.—Photo-micrographic Apparatus. (E. Leitz.)

In the photo-micrographic apparatus (Fig. 74) made by Charles Baker to the design of one of us the endeavour has been to embody the principle of the *geometric slide* throughout, so that extreme rigidity is obtained. There is little liability of alteration of any of the parts of the apparatus as the result of climatic change, and, owing to its rigidity, the whole apparatus would move together if subjected to any shake or vibration.

The base consists essentially of two iron castings made from two patterns, a pair of each casting being used. These are designed on girder principle, each part of the supporting castings may be resolved into a triangle so that the utmost stiffness is obtained. The two castings carrying the camera portion are braced together at each end and midway by bolts and nuts.

The swinging base-piece carrying the microscope and illuminating apparatus is also a pair of castings, these being triangular in shape, and again braced together in the same manner as are the main castings. This unit is supported on a metal cone at the end away from the camera, which allows it to be swung out of alignment with the rest of the apparatus in the manner already described in other designs of photo-micrographic apparatus. The female part of the cone is on the swinging base-piece, whereas the male portion is carried at the end of the main castings. The other end of the swinging base-piece is supported on a segmental arm and rests on two points. It therefore satisfies the requirements of the *geometric slide,* in so far as it has one degree of freedom only allowing it to rotate on a vertical axis. It is, therefore, supported at its centre on the cone, and its two other points of support are quite free. No shifting of the part is possible other than in the direction required, and it differs essentially in that respect from other arrangements, where a bolt or metal pin is used as a centre of rotation.

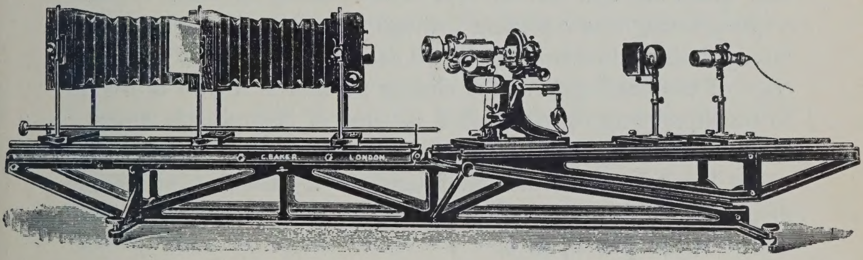


FIG. 74.—Photo-micrographic Apparatus. (C. Baker.)

On the top of the main castings two stiff metal rods are placed. These rods are fastened down firmly to the castings, and the camera slides to and fro on two V-grooves on one side and on a plane surface on the other in the manner already described (see page 129), both the front, back and middle of the camera being so supported. On the portion of the apparatus carrying the illuminating arrangement and the microscope, the same method is adopted; the microscope is carried on a cast-iron base-plate, the necessary V-grooves and plane surface being all in one casting from one pattern. No more work is done to this part than to file off the roughness of the casting from the inside surface of the V-grooves, and from the plane surface on the opposite side. The plates carrying the illuminant, any subsidiary collecting-lens, water-trough, colour-screens or cells, are all supported in the same manner. Each has a quick-acting clamp rigidly fastening it down in any required position, and a screw is provided for centring illuminant and collecting-lenses.

The clamp is of a simple character, and consists of a metal bar, which engages on each side of the casting on which it rests, a quick-acting screw passing through it and the base-plate. On manipulating the screw the bar is drawn by it against the inner edge of the casting, and the sliding portion is drawn down on to its supporting rods. Such a method of clamping is superior to that in which subsidiary appliances are carried on a triangular metal bar. In the latter the clamp is at the side, and some displacement is unavoidable if the clamping screw has any undue strain put upon it.

The camera, which is half-plate size ($6\frac{1}{2}$ inches by $4\frac{3}{4}$ inches), with 3 feet extension, is supported on vertical rods firmly fixed to the *geometric slides* already described. This allows vertical movement of the camera to suit microscopes of different sizes and of any optical height. The camera can be raised or lowered to suit the required conditions. There is nothing special about the camera portion as such, except that on one side a small white screen is supported, so that when the microscope, illuminant, etc., are thrown out of alignment on the swinging base-piece, an image may be projected on this small subsidiary screen. The preliminary adjustment of the instrument, either in the way of centring the sub-stage condenser, the illuminant, or any subsidiary condensing systems, as well as altering the position of the object itself, may be effected by directly observing the projected image. Final focussing must, of course, be done on the ground- or clear-glass screen in the usual manner, but the advantage of this additional screen is considerable.

The apparatus is designed to be as far as possible *universal* in its applications. It may be used as a horizontal or as a vertical camera; it may be inclined at an angle of 45°, should such for any reason be required; it may be used in a manner indicated later for metallography, in which the source of light is usually placed at right angles to the direction of the optic axis of the microscope. It may conveniently be used for photographing large specimens which must be in a horizontal position, or in photographing other objects which are conveniently supported in a vertical position. For the latter purposes the swinging limb can be removed, together with the microscope and accessories, and a suitable photographic lens placed in the front panel of the camera. Alternatively the swinging tail-piece can be left in position, the microscope and accessories removed, and the object to be photographed disposed upon it.

For use as a vertical camera, the camera portion is carried on a subsidiary iron frame, which rests inside the main supporting castings. This frame is hinged at the end near the microscope so that it can be swung upwards and supported vertically. To ensure rigidity while in this position an additional strut is provided which clamps on the side of the apparatus, so that the camera itself is supported on the vertical iron frame, which is again braced to the main castings. In this case it is obvious that the position of the camera in relation to the microscope would require some alteration, and the manner in which the camera slides up and down on its metal rod supports provides for this. The illuminating appliances remain in the same position as when the apparatus is used horizontally, but the standards carrying these are provided with hinged joints, so that the illuminating beam can be projected on to the mirror of the microscope when the latter is vertical.

For metallography with this apparatus the method adopted is to use it in the horizontal position, and to mount a totally reflecting prism (Fig. 75) over the vertical illuminator attached to the microscope. This prism is mounted above the stage of the microscope, when the latter is horizontal, high enough to receive a beam from the illuminant and from any subsidiary condensers without obstruction. This arrangement appears to be more simple, and certainly involves less alteration in any apparatus, than placing the illuminant at right angles to the optic axis. All adjustments are quite as easily made, and the proper illumination of the object can easily be carried out by observing the reflected image on the subsidiary screen already described.

No unusual arrangement is suggested for controlling the fine-adjustment of the microscope at a distance as any recognised method may be adopted. The one supplied is very simple, on well-known principles. A central metal rod having a large milled head at the operating end is pushed into contact with a pair of bevel wheels at right angles to one another and fixed to the microscope base-plate. These wheels, being provided with grooves, may be connected by a waxed silk thread, or by any other suitable arrangement, with the fine-adjustment milled head of the microscope, whether this be at the side or back of the instrument.

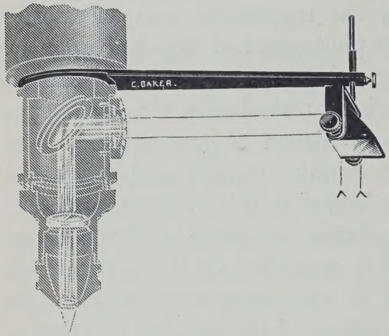


Fig. 75.—Totally reflecting Prism as arranged for Metallographic Work. (Baker.)

The base-plate designed to carry the microscope is so arranged that almost any type of instrument, whether on a horse-shoe or tripod foot, may be used at will. A clamp is provided which renders it possible for the microscope to be fastened down.

The position of the microscope on the base-plate is determined by a slotted right-angled piece of brass, which may be fixed in any position. The microscope is centred on the base-plate and then clamped down, and there is sufficient range of adjustment to enable it to be centred once for all.

All the subsidiary apparatus carried on the *geometric slides* on the swinging tail-piece, are mounted so that they may accurately be centred to the optic axis of the microscope, and clamped down when their position has been determined.

The recent development of small photographic cameras using fine-grain films has resulted in the design of similar apparatus for photo-micrography. No doubt very good results can be obtained, but the method has definite limitations. The negatives obtained are small in size, usually about double the size of a professional cinematographic picture, and are subsequently photographically enlarged. A typical example is the Leitz 'Micro-camera Attachment' (Fig. 76) which essentially is a roll-film holder made for attachment to a microscope. Thirty-six pictures can be taken consecutively with one loading of special roll film, each picture measures 36 × 24 mm. The focussing device is similar to that described for the 'Micca' camera.

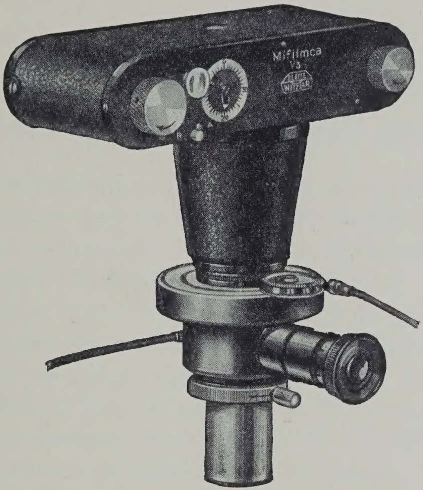


Fig. 76.

The well-known Leica camera can be adapted for a similar purpose.

There are many other types of photo-micrograph cameras, but those described cover in the main the best-known forms. Most optical firms now make such apparatus and their catalogues will contain all essential details. It must be recognised that any suitable combination of microscope, camera, illuminating apparatus, and illuminant can constitute a photomicrographic equipment. So long as these essentials are so disposed that accurate adjustment of each element can be secured there is no limit to the class of work to be done.

Success is not dependent on elaborate apparatus but on the use of good quality optical parts in accurate adjustment. Simple appliances therefore can, and in fact usually do, give results in no way inferior to those produced by elaborate equipment.

CHAPTER VII

THE USE AND MANIPULATION OF THE MICROSCOPE

Essential Adjustments.—It is, above all things, necessary that the microscope and its optical parts should be in perfect adjustment, as on this more than on any elaboration of apparatus will depend the excellence of the results. It does not come within the scope of this work to go fully into the theory and practice of microscopy, and reference must be made to one of the standard works on this subject if fuller information is required. Sufficient will be said, however, to enable anyone who already has some knowledge of the microscope and its use, to make the necessary adjustments. The optical portion of the microscope consists essentially of the three parts already enumerated :

The objective, which forms the actual image of the object ;

The eye-piece or ocular which more or less amplifies this image ;

The sub-stage condenser, for efficiently illuminating the object.

It will be assumed that some simple uncoloured object presenting no particular difficulty is placed upon the microscope stage. A low-power objective—for example, a 1-inch—should be screwed into position on the instrument. The sub-stage condenser had better for the moment be removed and illumination of the object effected by reflecting light from the plane side of the mirror. The illuminant may be placed in a convenient position—say, some eight to ten inches away from the mirror—and the latter adjusted so that the light is reflected on to the object. On observing the image through the eyepiece of the microscope an evenly illuminated field should be seen if the mirror is so adjusted that the light passes centrally up the microscope tube.

In any case, before taking a photograph this operation with greater or less elaboration will be necessary, so that the part of the object required in the field of view may be brought into position and illuminated satisfactorily.

In observing the image, if the worker has not already accustomed himself to do so, he should at once endeavour to train himself so that both eyes always remain open. This applies not only when observing the image visually, but also when focussing, the image being projected on the ground-glass screen of the camera and observed with a focussing ocular. At first this may seem a trifle difficult as extraneous objects become painfully evident. But it will be found that it soon becomes an easy matter if the eye not in use is for a time partially shaded with the hand or with a piece of black cardboard. Both eyes should be used alternately, so that there is no greater strain on one than the other. Where long searches have to be conducted it is a great relief to change from one eye to the other at short intervals, thus reducing the risk of fatigue.

It is assumed that there is no great optical difference between the two eyes of an observer ; if there is such a difference it is almost certain that he will be wearing corrective spectacles which should not be removed. Perhaps the case of one of the writers will be illustrative. He wears glasses which correct the usual errors and provide him with two nearly equal normal eyes. But the ability to appreciate fine detail is greater with the right eye whereas the recognition of colour differences is easier with the left.

The object having been set up, the most instructive procedure is to turn the microscope into the horizontal position and, instead of viewing the image directly, to project it on to a small white screen, such as a piece of white cardboard some ten inches square, placed at a distance of eighteen or twenty inches from the ocular. The mirror must of course either be swung aside or removed. It is with projected images that the worker is almost entirely concerned, and some preliminary experiments in simple projection will be far more useful than to begin by exposing plates.

When the microscope is in the horizontal position, assuming that the object has been approximately focussed and the source of light placed so that it is properly illuminated, the projected image will be seen on the white screen placed as suggested. It may be found that some alteration in the position of the illuminant will be necessary before the field becomes evenly illuminated. It will somewhat facilitate perfect centration if the iris-diaphragm on the microscope be now closed, so that only a small point of light is allowed to pass. Should there be any want of centration in the position of the light, this will now become evident, and a much smaller error is at once seen if the iris is closed than when it is wide open. Having secured centration the iris must be opened, but only sufficiently to well illuminate the object. The bull's-eye condenser, or a similar auxiliary collecting-lens, may then be interposed between the source of light and the object. It is important to see that this lens is placed so that it is exactly at right angles with the optical axis both in a vertical and horizontal direction. It will be found that it is in its best position when either an approximately parallel or a convergent beam is projected on to the stage of the microscope, or on the iris-diaphragm underneath the stage. Some to and fro movement of the bull's-eye condenser will no doubt be necessary to obtain this result. The condition, therefore, is that the illuminant is approximately at the principal focus of the bull's-eye condenser, which should present its plane side towards the light, and the object is evenly illuminated with a parallel or slightly convergent beam. The iris-diaphragm on the microscope should then be contracted until no more light than is necessary reaches the object, and so that the size of the illuminated area is no larger than is required to illuminate the field of view. It should be noted that to obtain a parallel beam the bull's-eye must be near to the source of light, and must be moved away from the light to obtain a convergent beam. It will be useful to alter the position of the light or of the bull's-eye in relation to the microscope and watch the unevenness of illumination that results from any want of centration. It is on this point more than on any other—the correct centration of the light and auxiliary collecting-lens—that good results depend in low-power work.

It is assumed that the worker is conversant with the appearances of an ordinary projected photographic image, such as may be seen in any ordinary camera with an ordinary photographic lens. If he is not, then the preliminary work had better consist of the observation of such an image without a microscope at all, but with an ordinary photographic lens on the front of his photo-micrographic camera. He may then take any large microscopic object and perform the same operation of illuminating the object and obtaining an evenly illuminated picture, as already described with a low-power objective on the microscope.

This experimental method of observation of the projected microscopic image may then be extended, and a rather higher power objective put on to the microscope—say, a half-inch—the sub-stage condenser replaced, and the illumination watched as before. The upper lens of the sub-stage condenser should be removed, if its construction will admit, so that the image of the illuminant is larger and so covers a larger field than it would were the condenser complete.

The illumination in this case may best be effected by removing the bull's-eye for the moment, then racking up the sub-stage condenser until an image of the illuminant is seen projected over the image of the object. The illuminant should for this purpose be about ten inches from the microscope sub-stage, and its image should be seen in the centre of the field. Then replace the bull's-eye condenser, and arrange it as before in such a position that the field again becomes evenly illuminated. The effect of altering the position of the bull's-eye between the light-source and the microscope should be noted; but the change should be effected by moving the bulls-eye from the position in which it gives a parallel beam, towards the microscope so that it gives a converging one. To obtain an evenly illuminated field it is necessary for an image of the aperture of the bull's-eye itself to be projected into the object-plane. In cases where the bull's-eye has an iris-diaphragm or a single metal plate with a small hole in it—an accessory often supplied with these appliances—by racking up or down the sub-stage condenser it is easy to focus the image of this small aperture in the same plane as the image of the object. On removing the stop or opening the iris-diaphragm the object then will appear evenly illuminated. If a parallel beam from the bull's-eye does not give enough light, a convergent beam may be used; but it should be arranged so that a roughly focussed image of the radiant is seen on the iris-diaphragm at the back of the sub-stage condenser. The illumination will then be suitable for an objective not shorter than half an inch in focal length.

Further reference to this and instructions in detail will be given later when referring to the photography of typical objects; but this preliminary observational work will be found most useful in enabling one not conversant with the appearances obtainable to appreciate the effect of altering the position of the various parts in use.

The Use of Objectives and Oculars.—In ordinary observational work the use of the low-power objective presents no particular difficulty; it is only necessary to screw the objective into its proper position on the microscope body-tube, in conjunction with a suitable eye-piece, and focus the objective on the object, when an image will appear. As a practical point, it is advisable with low or medium powers to rack down the objective with the coarse-adjustment, so that its front is in close proximity to the object. To bring the object into focus the motion of the body-tube is therefore away from the object. This avoids the possibility of injury to the specimen or the objective by racking down too far by means of the coarse-adjustment. With high-power objectives this is hardly practicable, as the working distance is already so small that to bring the objective still closer is a matter of considerable difficulty. One way therefore is, having if necessary immersed the objective, to carefully focus until some particle of dust on the upper surface of the cover-glass is seen, and then by means of the fine-adjustment to work down until the object is in focus. It is more satisfactory to place the immersion medium on the front of the objective itself, and then to lower this down on to the object, than to place it on the object-slide as is so generally done. This is perhaps a small point, but it ensures that the immersion medium is in the proper position on the object, and it prevents the possibility of too large a quantity being used. When the microscope is in the horizontal position if there is any considerable excess of oil it tends to flow away; should this occur during an exposure, the photographic result would be spoilt.

It is necessary to see that the drop of oil or other immersion medium does not contain air-bubbles, a frequent cause of difficulty in obtaining a satisfactory image. If the oil is air-free in its container, the inclusion of air during the process of immersing may be prevented by putting a very small drop on both objective and object-slide. The convex surfaces of these two drops meet when the objective is lowered and so prevent the entrance of air-bubbles.

Before inserting an ocular into the body-tube it is important to see that the tube-length is that for which the objective has been designed. As explained later, some variation of tube-length may be necessary; but for low power objectives, or for immersion objectives, it will not be advisable to depart from that stated by the maker to be correct. The majority of objectives are now corrected for either 160 or 170 millimetre tubes; and most microscopes have sufficient available length of tube to accommodate themselves to the required condition. It is important to remember that in adjusting this tube-length due allowance must be made for any objective-changer that may be on the microscope. The only satisfactory way when purchasing any such appliance is to ascertain the actual allowance that it entails.

In practice it is desirable to prevent any dust or dirt reaching the objectives, and when one is removed from its case, both the box portion and the lid of the case should be inverted on the table to prevent the entry of dust. A well-washed piece of linen or silk should be kept specially for cleaning purposes. It is advisable to keep a small piece in a stoppered bottle or some such closed receptacle, and to take it out only when required, replacing it immediately after use. To clean immersion objectives a very slight moistening of the rag with xylol or benzole may be recommended. Any excess of either of these solvents is carefully to be guarded against, as it may injure the objective if it reaches the inside of the lens mount. An even better material for cleaning purposes is Japanese silk paper; a small piece of this may be torn off when required, and can be thrown away after use so that no dirt can accumulate on it.

For observational work the selection of the most suitable ocular, and the advisability of using a low or high-power one, is an important matter; but in photo-micrography any but low-power oculars may, except under special circumstances, be excluded. It may be necessary, where great amplification is required, to use one of high power; but this will only be found satisfactory when the objectives are apochromats, and the oculars therefore of the compensating type. If an ordinary ocular is used, no adjustment of its parts is necessary, but with projection-oculars it is important to see that the image of the diaphragm inside the ocular itself is sharply projected on the focussing-screen of the camera. This procedure has been sufficiently described in the section dealing with the various types of oculars. When no ocular is used in conjunction with the objective, the inside of the body-tube of the microscope must be completely blackened; otherwise internal reflections are sure to result, and a central flare-spot will appear on the focussing-screen of the camera. This is most effectively provided against by inserting a cardboard tube lined with black velvet, and of such a size that it slips into the body-tube; it should be long enough to come quite flush with the top of the latter.

Achromatic objectives that may give a good visual image are not always corrected to give a sharp photograph in the same plane. The reason is that achromatic lenses are corrected for the yellow and green rays only—that is, for the portion of the spectrum of the greatest visual luminosity; but they are not corrected to utilise the less luminous blue and violet rays. With apochromatic objectives this does not apply, and these should give a perfectly defined image whatever the illumination may be. One method of overcoming the difficulty with achromatic objectives is to ascertain the difference between the visual and the actinic focus. This can be ascertained by taking a series of photographs with each lens, and measuring the alternation in the focus necessary to secure a sharp photograph. If the fine-adjustment on the microscope is graduated this is not a matter of great difficulty; it is only necessary to determine the number of divisions through which the micrometer screw has to be moved to obtain correct photographic focus, and to alter the fine-adjustment by this amount every time a photograph is taken. It is assumed that the object is focussed visually beforehand on the ground-glass screen of the camera, and this allowance made. Obviously this is a somewhat laborious matter, and it is by no means certain that under all conditions the focussing will prove to be as good as might be desired.

The better method is always to use a colour-screen with achromatic objectives. This does not increase exposure very substantially, as it only requires to be of a pale-yellow or pale-green colour—one that will cut out effectively the blue and violet rays. Or again, if the screen is approximately monochromatic, whatever colour it may be, the object when focussed visually will nearly always be found in correct focus photographically. The best screens to use for this purpose will be given in Chap. IX of this book, which treats of the use of colour-screens in general. It will be obvious that it is better to use apochromatic objectives, since these colour differences do not arise; although it must be admitted that often where the necessary trouble has been taken to secure a correct colour-screen the difference between the results obtained by using either apochromatic or achromatic objectives can hardly be appreciated.

It is well to remember that dust on either of the components of the ocular will become evident in the photograph. For this reason it is necessary to ensure that the oculars are kept perfectly free from any dirt. If dust is evident in the field of view, it can be determined at once whether it is on the ocular or in the object, by rotating the ocular itself. If it is on any part of the latter, the image of the dust particles will rotate. Steps may then be taken to remove it, but the cleaning should be as carefully performed as in the case of an objective. If it is necessary to unscrew any part, it is essential that it be firmly screwed up again to its correct position.

When using illuminants of high power, no attempt should be made to observe the image in the microscope directly, unless dark glass or some similar light modifier is placed in the path of the illuminating beam. The advantage of carrying out the various adjustments by means of the projected image on an opaque screen again becomes evident, as there is no possible risk of any harm to the eyes. This is a point that requires consideration when working with illuminants of the type of the electric arc, or of the quartz mercury-vapour lamp. From both of these a considerable emission of ultra-violet light takes place and this, even with an exposure of only a minute or two, will cause painful and even serious inflammation to the eyes. Illuminants should in all cases be efficiently screened so that no direct light reaches the observer.

Correction for Thickness of Cover-glass and Adjustment of Tube-length.—A point of great importance in using high powers—particularly those other than immersion objectives—and one which affects the quality of the image to a very marked degree, is the thickness of the cover-glass over the object. A flat refracting surface behaves to some extent like a spherical one; in fact, the flat surface may in theory be regarded as a spherical surface of infinite radius of curvature. It therefore suffers, although to a less extent, from the same defect as such a curved surface; oblique marginal rays are refracted to a greater extent than those situated nearer the centre. If an object is mounted in Canada balsam, light emitted from any one point in the object (when it emerges from the surface of the cover-glass into air) would proceed not as if it were coming from one point only, but from a succession of superimposed points. The effect would be, therefore, that the rays of greater obliquity would appear to emerge from a different point from those that were less oblique. Fig. 77 diagrammatically illustrates this point. Let the horizontal line FG represent the top surface of a cover-glass, the object being mounted in Canada

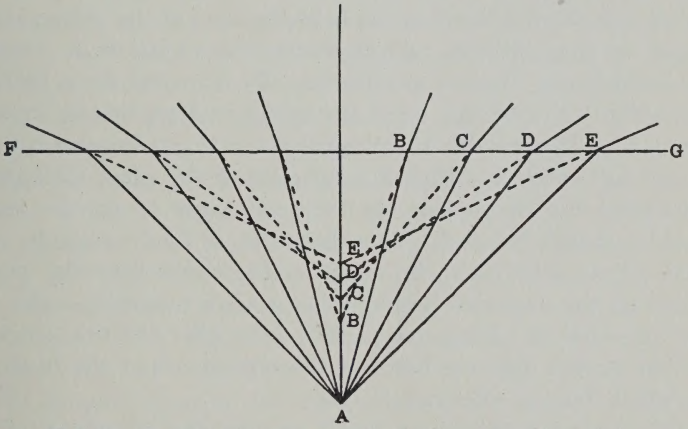


Fig. 77.—Influence of Cover-glass Thickness on Adjustment.

balsam beneath it. If light were being emitted from a point in the object A, it would appear not as if it were from A only, but also as if it were emitted from the points B, C, or D, according to its obliquity. This example is, of course, hypothetical and for purposes of demonstration exaggerates the actual conditions. The practical effect is that a cover-glass introduces an aberration which the objective has to correct. If the cover-glass is thicker or thinner than the particular thickness for which the lens is corrected, it influences the performance of the lens; the latter then would not give as good an image as when working under proper conditions. In the case of apochromatic lenses, particularly the 4-mm., and usually in the best quality achromatic lenses of about one-sixth inch focal length provision is made, by means of what is called a correction-collar, for altering the distance between some of the component lenses of the objective to compensate for variation in cover-glass thickness. Lenses are now usually corrected for a thickness of 0·17 to 0·18 mm., and any variation from this thickness introduces an error due to spherical aberration. To obtain the best results with any lens, a cover-glass of the exact thickness stipulated by the makers, in their catalogue or on the lens mount, should be used. This, however, is rarely possible, so that adjustment has to be made by the correction-collar provided on the objective itself. When such a correction-collar is not provided an alternative method is to alter the tube-length—that is, the distance between the objective and the ocular, to which further reference is made.

Oil-immersion objectives do not require any adjustment for this variation in thickness when the object is mounted in a medium of approximately the same refractive index as glass, for under these conditions no refraction of the light occurs at the surface of the cover-glass. When work of a critical nature is being carried out, particularly when the resolution of the structure of diatoms mounted in media of high refractive index is attempted, this does not apply. The correction necessary in these cases, however, is usually very small and such that it may be effected by alteration of tube-length; in this class of object great care is exercised and considerable experience is necessary in making a preparation, and it is usual to calibrate the cover-glass before mounting. An oil-immersion objective is not so sensitive as a dry lens to any small departure from the required conditions. The advantage of the dry lens lies in its greater focal length, and if the necessary magnification can be obtained with it, the additional working distance is helpful. With objects mounted beneath a thick cover-glass a dry lens is the only possible one; in such cases the necessary correction must be made by the correction-collar, or the tube-length must be shortened.

The adjustment of a lens by means of a correction-collar by observation is an easy matter to a microscopist of skill and experience. It is by no means so easy for a novice, yet it is the best exercise in practical microscopy that any worker can have. It is obviously impossible accurately to measure the thickness of a cover-glass on a mounted object; but where a microscope possesses a graduated milled head to its fine-adjustment, an approximate estimate of its thickness may be obtained without much trouble. If the objective in use also has marked on it the correct position of the index on the correction-collar for a given thickness (as it should have), it is then only necessary to set the collar for the determined thickness. If the specimen be an ordinary bacteriological cover-glass preparation, all that is required is to focus a bacterium in the preparation, and to raise the objective by the fine-adjustment until some small particle of dust on the top surface of the cover-glass comes into view. The difference of focus, as shown by the graduation of the milled head, will then indicate the relative thickness of cover-glass in use. To ascertain the actual thickness of the glass, the distance so found is multiplied by the refractive index of the glass (approximately $\frac{3}{2}$), and this would give the approximate thickness of the cover. It is, however, clearly of the nature of a makeshift method since no allowance is made for possible variations in the thickness of different parts of the same cover-glass—a factor by no means negligible—or for the cases in which the part of the object in view lies at a greater or less distance below the surface. It is not nearly so satisfactory as direct observation of the effect that collar-adjustment or alteration of tube-length has on the image itself. It is not always recognised that the thickness of the object may have as much as or even more influence on cover-glass correction than variation in the cover-glass.

Reference to Fig. 78 *a* and *b* will enable a better idea to be obtained of the way in which correction is effected. Fig. 78 *a* shows the path taken by rays of light which come from a perfectly corrected lens system, and which form an image so that all the rays pass through one point. Fig. 78 *b* shows the image formed by a lens system that is not in correct adjustment, and in which the rays do not come to one point. It is evident from Fig. 78 *a* that if the rays are examined at the position A or B, instead of at the point F, although the object will be out of focus the images given will be identical whether we examine them on one side or the other of the correct focus. In either case a disc of light will be seen, and these discs will be equal in brilliancy; the disc A will be exactly the same in every particular as the disc B. Such, however, is not the case with Fig. 78 *b*. The image in the position A will here be much more brilliant than in the position B; in fact, at B there will be nothing but a large and diffused patch of light. The images observed at the points A and B will therefore be totally dissimilar. This is the method actually adopted in correcting for a desired tube-length or thickness of cover-glass, and opticians when effecting this correction in the course of manufacture usually have some object with which they are familiar and of which they know the appearance under varying conditions. On focussing such an image first above and then below the focus, and successively performing this operation and comparing the two appearances, it can be determined whether the objective is properly corrected for the conditions under which it is to be used. It is not by any means easy

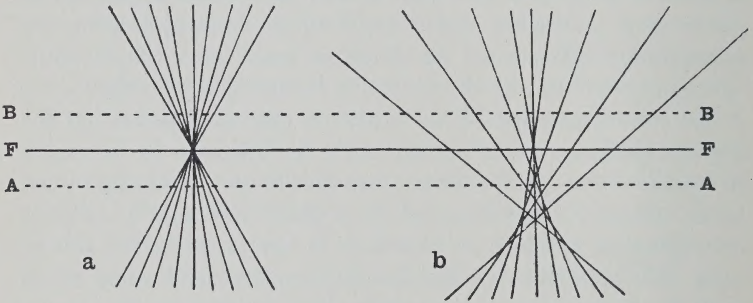
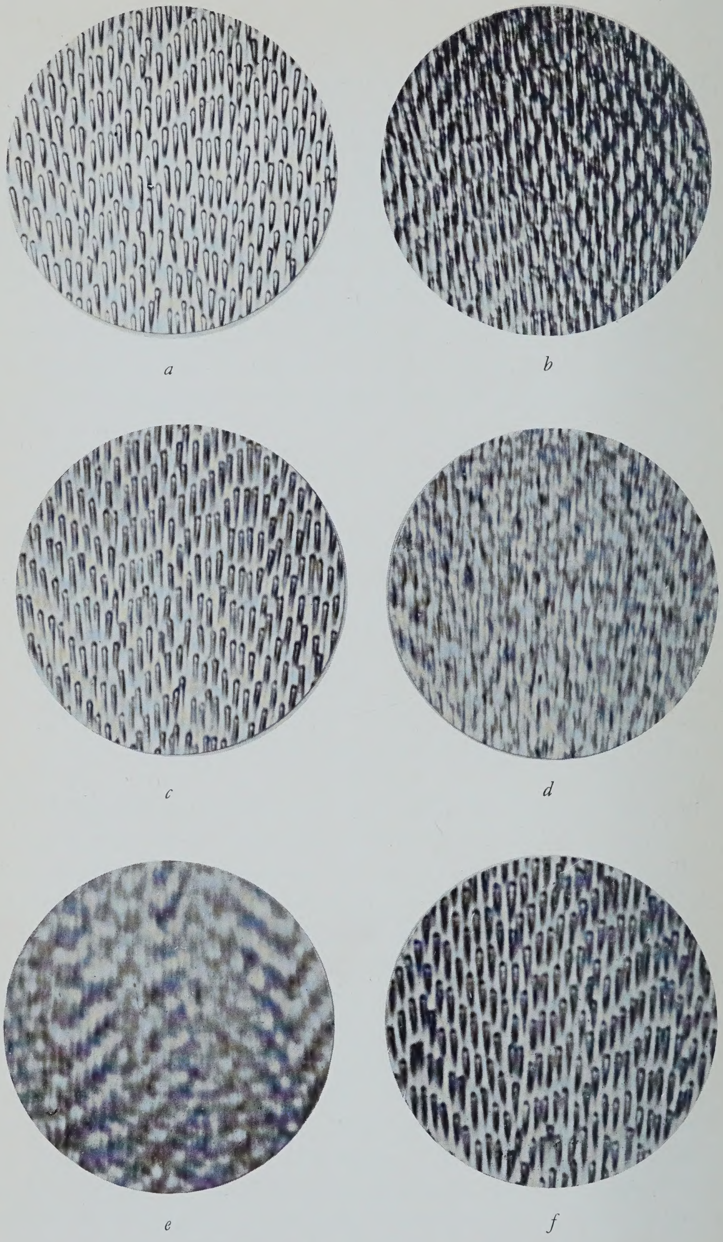


Fig. 78.—Influence of Cover-glass Thickness on Adjustment.

PLATE V



Effect of tube-length correction on the image of a Podura Scale.

for a novice to appreciate the differences and to get correct adjustment; a good deal of practice is necessary, only the experienced microscopist can obtain the best correction in actual practice. When working with an object which has a cover-glass of unknown thickness it will be found best to focus, and to effect the correction by means of some small dust-particle in the object. On focussing this, both above and below its proper focal point, the difference in the images will become apparent. The objective must then be adjusted by its correction-collar, if it has one, or by altering the tube-length of the microscope until the image appears exactly the same whether the focussing is above or below the proper focal point. Alternatively, any fine line or hair in the object may be utilised to perform the operation. If the lens is not properly adjusted, the appearances on opposite sides of the true focus will not be identical.

The best object for demonstrating the effect is the scale of a Podura (*Lepidocyrtus curvicollis*). This is usually employed by opticians to correct their objectives both for thickness of cover-glass and for length of tube. Plate V shows at *a* the appearance of a portion of the scale that may be expected if it is in correct focus and the lens perfectly adjusted; at *b*, the appearances well outside both sides of the focus when the adjustments are correct, and at *c* the appearance as it is just going out of focus on either side; *d* and *e* show the two distinct appearances, one on either side of the focus, when the objective is not in adjustment; *f* shows the nearest approach to a sharp image that can be obtained with a lens not properly corrected, or not in correct adjustment for thickness of cover-glass.[1]

The reason why the Podura scale is such a good test, is that the appearances on either side of the focus are of different patterns when the lens is not properly corrected; when the lens is perfectly corrected this is changed into one and the same pattern whether the lens is moved either within or without the focus.

It is unfortunate that at present good mounted specimens of Podura scales are difficult to obtain, and even these are expensive. An alternative is the well-known diatom *Pleuro-*

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[1] See Cantor Lectures on *The Theory of the Microscope*, by Conrad Beck.

sigma angulatum. This object requires a good deal of practice to get the best appearance, and in some respects is somewhat misleading as the appearances that can be obtained are so varied. Select one mounted dry and with a frustule that is in optical contact with the cover-glass; if the frustule is itself fractured so that a broken edge is in the field of view it will facilitate correction. Carefully focus this broken edge so that it shows what is usually referred to as the postage-stamp fracture. If the correction for cover-glass thickness and for tube-length is as it should be, then on getting this edge exactly into focus the slightest possible movement of the fine-adjustment will bring into view a perfectly defined black-dot pattern. If either of the conditions named are departed from, this black-dot pattern will only appear if the fine-adjustment is moved to a considerable extent, either above or below the point at which the broken edge is in focus. If it is necessary to focus downwards to get the black-dot image into view, it indicates that the tube-length must be increased; or alternatively that the correction-collar on the objective must be altered in the direction which would be required when the thickness of the cover-glass is less than that for which the objective is corrected. On the contrary, if it is necessary to focus upwards, the opposite conditions are indicated; and in each case the tube-length or the adjustment by the correction-collar must be altered in the contrary direction. Perhaps for a beginner this object is even more useful than a Podura scale, for even with a brief experience of the appearance of microscopic images, the position of the image either above or below a particular focal point is quite easy to observe. It will be of educational value to watch the effect on other parts of the image, varying the two conditions referred to, as it will facilitate these adjustments being made on an object that does not lend itself to this particular treatment.

When such corrections are attempted the highest power eye-piece should be used that the objective will stand. In the case of achromats this will be an eye-piece that does not enlarge the image more than ten times, while with apochromatic objectives an ocular magnifying eighteen times is the most convenient to employ.

When possible, the object should be covered by a cover-glass of the correct thickness, and the lens used at the correct tube-length as indicated by the maker. Correcting for thickness of cover-glass by alteration of tube-length is not ideal, it is in the nature of a compromise.

It has already been mentioned that for many classes of work, particularly in biology, the necessity for using high-power dry objectives is now not so great. A one-sixth-inch achromat or 4-mm. apochromat is particularly sensitive to cover-glass or tube-length alteration. These objectives can be replaced by using an 8-mm. apochromat, which is not nearly so sensitive, and the necessary magnification can be obtained by using higher-power oculars or by increasing camera length. The difference in N.A. if any, is not significant. The introduction of one-seventh and one-eighth-inch oil-immersion achromatic objectives has really in large part supplanted high-power dry lenses, together with the need for cover-glass correction. The need for correct tube-length, of course, remains and is particularly important when these lenses are used for dark-ground illumination, for which purpose they are very suitable.

The Use and Adjustment of the Sub-stage Condenser.—When using medium and high-power objectives, a sub-stage condenser must be employed. This should preferably be carried in a centring sub-stage together with an iris-diaphragm. Various types of sub-stage condensers can be obtained and have already been described, so that it only remains to indicate the methods of use.

The full aperture of the condenser should approximate to that of the objective of the highest aperture. It will usually be necessary to close the iris-diaphragm to some extent to obtain satisfactory illumination; but unless the condenser-aperture nearly equals that of the objective, the full advantage of the latter cannot be obtained when the resolution of difficult objects is attempted. Most achromatic condensers may be used with medium powers if the top hemispherical lens is removed, as it usually can be; the remaining portion then forms a very satisfactory condenser, which can be used with such powers. To utilise the whole of the aperture of immersion-objectives, the sub-stage condenser must itself be an immersion one; but some of these also work satisfactorily if used dry.

In illuminating an object the first point is to ensure that the optical centre of the condenser coincides exactly with that of the objective. This may be determined by closing the iris-diaphragm as much as possible so that only a very narrow beam of light passes through. An objective should of course be on the microscope, and some simple object in focus. Rack down the condenser until the image of the aperture in the closed iris-diaphragm comes into view and is sharply focussed ; it will then appear more or less well centred in the field of view. By means of the centring-screws on the sub-stage bring this image of the diaphragm exactly into the centre of the field. It is advisable to slowly open the diaphragm until this image reaches the edge of the field. It should be so adjusted that it evenly disappears from view all round the visible field. The illuminant should approximately be centred before the adjustment is made, as otherwise it is not easy to get the whole of the outline of the diaphragm-aperture in correct focus ; in fact, if there is any falling off or haziness to one side or the other, it clearly indicates that the illuminant is not in its correct position. The preliminary operations will usually be carried out with the microscope on a table, and suitably inclined for visual work. This adjustment having been made, the condenser may be racked up until the image of the radiant comes into view. The diaphragm may now be opened, and the degree to which this should be done is a matter of considerable moment. The merest novice will soon perceive that, with a diaphragm that is closed to a considerable extent relatively to the aperture of the objective, a falling off in the quality of the image results. For ordinary purposes, and when observing pathological, botanical, or any similar preparations, the best result is usually obtained when the diaphragm is open sufficiently to fill approximately two-thirds of the objective aperture. The proportion of the objective aperture utilised may be determined by removing the ocular from the body-tube of the microscope and looking down the tube at the back lens of the objective. If the whole aperture is being employed the back of the objective will appear to be quite filled with light, but as the diaphragm is closed the circle of illumination decreases. The most satisfactory result is usually obtained when the diameter of the circle of illumination is equal to two-thirds of the diameter of the whole visible aperture of the back lens.

There are very few objectives which will stand a larger cone of illumination than this; but for bacteriological or similar preparations it will be found that a larger cone may sometimes be used. In photographing it is, from a pictorial as well as from other points of view, necessary to have an evenly illuminated field. This may often be obtained by racking the condenser up or down, and so departing from the conditions that give the best illumination. This is a method, therefore, that should never be adopted if any other means of attaining the same result are available. The object of the auxiliary collecting-lens is to get over this difficulty, as in effect it enlarges the image of the source of light. If for any reason the only method of obtaining even illumination is by altering the position of the condenser, it is better for it to be raised nearer the object-slide, so that the apex of the cone of illumination is above the object, than for the reverse operation to be performed.

Sub-stage condensers are always of short focus, and it is therefore advisable that in making these adjustments only medium-power objectives should be on the microscope; so that in the event of the condenser being raised too high and coming into contact with the underside of the object-slide, the latter is not forced up against the front lens of the objective.

It must not be overlooked that when using any form of objective-changer—such as a multiple nose-piece—the sub-stage condenser must be re-centred for each change of objective. It very rarely happens that these appliances are sufficiently correctly made to ensure the axis of any one of a series of objectives always coming in the same position. With the Zeiss objective-changer or any similar appliance this does not apply, as it is usual for the worker to carefully centre the whole series of objectives in use when the changers are fitted.

In centring, where a change of objective becomes necessary, the following method is satisfactory. Place on the microscope the objective with which the photograph is to be taken, presumably a high-power one. The object on the stage may conveniently be a glass slip on which two diamond lines or scratches at right angles have been made, this being the most convenient object to use for all preliminary centring purposes. Arrange this object on the stage so that the point of intersection of the diamond scratches appears exactly in the centre of the field.

Now remove the high-power objective and substitute for it one of medium power, and carry out the necessary operations to obtain correct centration, as already described; but, in this case, the illuminant and condenser must be centred not to the centre of the visible field of the objective, but to the point where the images of the diamond lines intersect, since this is the position of the optical centre of the objective to be used. The centration will therefore be correct when the high-power objective with which the photograph is to be taken is replaced. Assuming that the illuminant and any auxiliary collecting-lens have been carefully centred for one objective, it is not necessary to alter their position when effecting a change of objectives. The differences of centration in the latter are usually not so great as to render it necessary to alter the position of the illuminant, re-centration of the sub-stage condenser being sufficient to effect the desired result.

It is assumed that the adjustments so far described have been made, or at least that there has been some preliminary practice of the process with a microscope on a bench and not on the photo-micrographic apparatus. The centration of the object and the sub-stage condenser having been effected, it remains to place the microscope in a horizontal position on the photographic apparatus, and the illuminant in position at a suitable distance from the microscope. If no auxiliary condenser or bull's-eye is used, this distance will be about ten inches. Probably it will then be found that the image of the illuminant is not in the centre of the field. To watch this image through the microscope and alter the position of the illuminant so that it is exactly central is usually a somewhat troublesome process, as it is generally difficult to reach the source of light while observing the microscopic image. It is assumed that the microscope is carried on a revolving table, so that it can be thrown out of the optical axis or that the camera itself may be moved back to allow of direct observation of the image. If the apparatus is of the former type, allowing of the movement of the microscope and illuminant as a whole, the great advantage of the small subsidiary opaque screen, set at the side of the focussing-screen of the camera as already described, will now become apparent. It makes it possible to project the image on to this opaque screen, and exactly centre the image of the illuminant to the centre of the field. In fact, given a sufficiently powerful source of light, it is by far the best method to make all the adjustments, centring the condenser and the illuminant, getting the correct position of the object in the field, and closing the iris-diaphragm to the required aperture, by observing the projected image. An illuminant of high power is not necessary for this purpose. A small arc lamp taking four amperes will enable adjustments to be made even if an object is being magnified as much as two thousand diameters. It is easier to exactly centre the optical components and the illuminant with a large image projected to a distance, and with the operator free to adjust any of them with facility, than to do it while observing the image in the microscope.

To prevent confusion it may be wise to recapitulate here the exact order in which the operations described should be performed :

1. Set up and centre the object with the objective with which the photograph is to be taken.

2. Remove this objective, if it is a high-power one, and substitute for it a low or medium power.

3. Close the iris-diaphragm in the sub-stage condenser as far as possible.

4. Rack down the sub-stage until a clearly defined image of the opening in the iris-diaphragm is seen in the field.

5. Adjust this image of the iris-diaphragm so that it is in the centre of the field ; or alternatively, where a change of objective is necessary, so that the image is exactly projected over the point of intersection of the diamond lines on the slide already described, or on any known central point in the object. Open the sub-stage iris-diaphragm to the required aperture.

6. Rack up the condenser until an image of the illuminant is exactly focussed in the field of view, the object itself also being in correct focus.

7. Centre the image of the illuminant by moving the source of light so that it falls exactly across the microscopic image. The term illuminant means either the image of the radiant or the image of the aperture of any collecting-lens.

When the illuminant is of large area, and where therefore its image more than fills the field, a good plan is to place a metal plate with a small hole in it as near as possible to the illuminant, so that only the centre of the latter is exposed to view. Then centre the image of the aperture in the plate—this, in fact, being treated as the source of light. This will only be necessary when a large-flame oil lamp or an incandescent gas burner is used, as no other illuminant is of sufficiently large area to more than fill the field when using low or medium-power objectives.

All these adjustments are best effected with a low-power ocular. The operations are similar whether the whole of the sub-stage condenser is used—as in the case of high-power objectives—or only the back combination. The advantage of using a well-achromatised condenser having a large aplanatic cone will, during these operations, become evident. The image of the radiant or of the subsidiary collecting-lens, if one is used, should appear bright and well defined in the field, and that portion of the field not covered by the radiant should appear relatively dark.

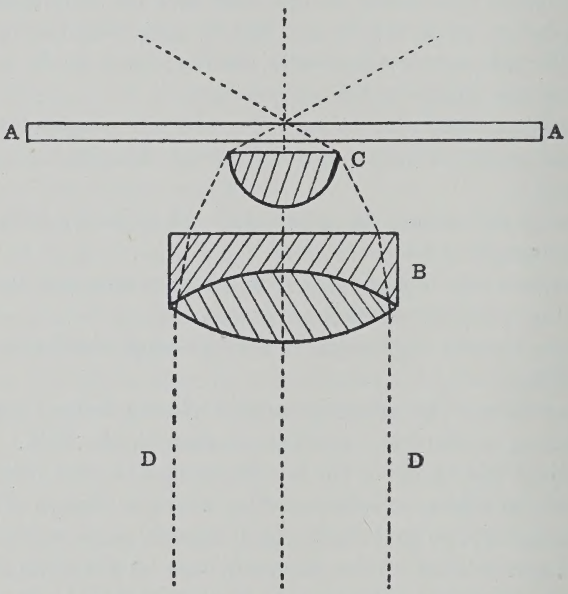


Fig. 79.—Diagram of Path of Rays in Sub-stage Condenser.

Fig. 79 shows diagrammatically the condition that should obtain when the condenser is filled with a parallel beam DD, and is focussed on the object lying on the slip AA. If the condenser is one with a full aplanatic cone—that is, one which is practically free from spherical aberration—the whole, or nearly the whole, of the light will be focussed on the object.

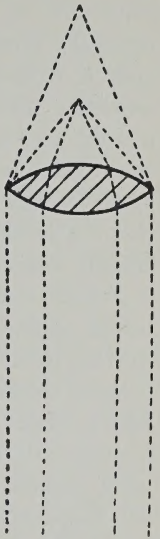


Fig. 80. — Diagram of Path of Rays in Sub-stage Condenser.

If the condenser is simply an uncorrected lens or is of the ordinary Abbe illuminator type, the central rays will be focussed in a different plane to the marginal rays (Fig. 80); and to get a clearly defined bright image of the radiant it will be necessary to close the iris-diaphragm somewhat, irrespective of what may be necessary to obtain a bright image of the object.

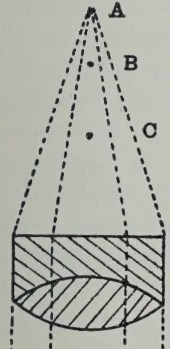


Fig. 81.—Diagram of Path of Rays in Sub-stage Condenser.

Not only is it impossible to obtain such a bright and clear image of the illuminant, but there is also a general loss of light owing to so much of the aperture of the condenser being used in condensing the light to a point where it is not utilisable by the objective. If the condenser is adjusted so that its focal point is either above or below the object, the effective aperture—as may be observed by looking down the microscope tube without the ocular—is less in proportion as the correct conditions are departed from. Fig. 81 may help to explain this. The points A, B and C may be taken to represent the position of an object in the cone of light formed by the condenser. Only at A does the object receive the benefit of the full cone, and at B or C neither the full amount of light nor the full aperture of the condenser is utilised.

A further advantage of the large aplanatic cone becomes evident when test-diatoms or similar objects have to be photographed with only a portion of the solid cone—that is, with annular illumination. In this case only a portion of the marginal rays transmitted by the condenser is utilised. The object therefore would not be correctly illuminated by a condenser having a small aplanatic cone if a stop with an annular opening were inserted; and assuming that the object had been previously illuminated with a solid cone some further adjustment would be required. In such cases a condenser of the highest order is essential: it must be well achromatised, and be free from spherical aberration. The shapes of the various stops that may be used have already been indicated, but in general it will be found that it is more convenient to improvise stops from thin black cardboard, cutting these to the shape and size required to bring out the details of a particular object.

Some workers are of the opinion that any auxiliary collecting-lens between the illuminant and sub-stage condenser is to be avoided. It may then be better to use an illuminant of somewhat greater area, to place it at its proper distance from the sub-stage condenser—usually a distance of seven to ten inches—and to focus the image of the radiant without any intervening optical system. The best arrangement is to use an achromatic auxiliary collecting-lens and to project a parallel or converging beam from a very small source of light, such as the electric arc, into the back combination of the sub-stage condenser. The writers have yet to find an object that cannot be perfectly illuminated by this method.

The Auxiliary Collecting-lens.—The advisability of the use of such a lens or lenses is a somewhat controversial point, and some workers of repute are of the opinion that no optical system should intervene between the source of light and the sub-stage condenser of the microscope. While for observational purposes this may hold, it becomes a matter of extreme difficulty to do without some such appliance in photographic work. The necessity for this is more particularly in evidence with a source of light of small area—such as the electric arc.

For very low-power work the auxiliary condenser may be replaced by a piece of ground-glass, and an enlarged image of the illuminant may be projected on to this. The ground-glass in effect therefore becomes the source of light, but this method, though simple, is not on optical grounds to be recommended.

The conditions with higher powers are that either the image of the source of light is enlarged by using an auxiliary lens so that it covers a larger proportion of the field, or the position of the radiant is so adjusted that the aperture of the lens itself is what is actually focussed in the field of view—the image in the field of view of the microscope appearing as a luminous disc.

Perhaps the most serious practical objection, and one that requires to be carefully guarded against, is that any intervening lens, unless exactly and properly adjusted, may reduce the working aperture of the objective; and it is against this contingency more than any other that the worker is warned.

The simplest lens of this type is the bull's-eye condenser, of which a description has already been given, and which is only suitable for use with low powers. A development of the bull's-eye system is the more highly corrected lens system devised by Mr. E. M. Nelson, and usually known by his name. This lens has already been described under the section devoted to such apparatus in Chap. IV, p. 79, as well as the Watson-Conrady system, the corrections of which satisfy the requirements of the most critical worker. In actual use all these lens systems are subject to practically the same working conditions, and it is therefore proposed to describe the method of adjusting the three-lens system already described as made by Messrs. Zeiss; these meet every need, are comparatively inexpensive, and are available for use with objectives of any power.

In conjunction with these lenses an iris-diaphragm on a separate stand is usually supplied, and in any case, whatever form of collecting-lens is used, such an addition is almost a necessity. A less expensive substitute is a series of metal plates, in which are holes of various sizes. These may be supported in any suitable carrier and are so arranged that they can easily be changed as required. The first point is to ensure that this iris-diaphragm or its equivalent is accurately centred to the optical axis of the microscope, and is in alignment with the centre of the camera back. To be quite certain that this is the case, it must be observed in relation to the sub-stage iris on the microscope, the optical portion of the sub-stage condenser having been removed. In some instruments there are means of de-centring this iris, the rack-work that enables the de-centration to be effected having a catch which indicates to the worker when centration is correct. With the usual English form centration of the iris would be effected by means of the centring-screws on the sub-stage. The operation may be performed by placing a low-power objective in the microscope, inserting an ocular, and viewing the image of the diaphragm directly. Any necessary alteration in the adjustment may then be accomplished by means of the screws on the sub-stage already mentioned. The ocular is next removed, and in its place is substituted a cap, which is perforated at its centre with a small hole about one millimetre in diameter. On observing the source of light through this pin-hole eye-piece and closed iris-diaphragm of the microscope sub-stage, it will at once be seen whether the illuminant is in correct alignment; unless it is no light will pass through to the eye. The illuminant having been adjusted centrally, the subsidiary diaphragm first mentioned may now be interposed between the sub-stage of the microscope and the illuminant. It facilitates any future adjustment if these extra fittings are carried on a triangular metal bar or geometric slide, as already described; but in any case, they should be so arranged that they may be removed and replaced in their proper position at will. The iris-diaphragm should now be closed as far as possible, and if all is in correct alignment on viewing the illuminant through the pin-hole eyepiece the closed sub-stage iris and the extra intervening diaphragm, a brilliant point of light should be seen. It is obvious that if there is any want of centration between these three small apertures, no light can pass, and it will be found extremely useful to devote some attention to this point to ensure that such adjustment is exactly carried out. Correct centration is the essence of these operations.

This having been done, the auxiliary condenser may now be placed in its position, which should be between the illuminant and the auxiliary iris-diaphragm, the sub-stage condenser having been replaced on the microscope. Assuming that the collecting-lens is the one described by Messrs. Zeiss by the Roman figure I, the iris-diaphragm should be placed near to it. If reference is now made to Fig. 36 *a*, it will be seen that when using this No. I lens a convergent beam is thrown into the back lens of the sub-stage condenser, and this should be a sufficiently broad beam to fill the back lens with light. This collecting-lens is of the uncorrected plano-convex type, and the same adjustment may therefore be made when using a bull's-eye or any other similar system.

An alternative method when a parallel or convergent beam is being used, is to place the subsidiary iris-diaphragm as near as possible to the collecting-lens, close the iris and focus its image in the field of view by means of the sub-stage condenser; the iris of course is opened again sufficiently to fill the field of view when a photograph is to be taken. An image of the iris is obtained therefore instead of an image of the illuminant. For high powers this lens No. I is sufficient to obtain a well and evenly illuminated field, even with an illuminant of the smallest area such as an electric arc of small current-consumption.

For medium powers the arrangement is seen in Fig. 36 *b*. In this the No. I lens is taken much closer to the source of light, so that a parallel beam is projected. The lens No. II is then placed in such a position that it takes up this parallel beam and focusses an image of the source of light on to the iris-diaphragm of the sub-stage condenser of the microscope. In this case the auxiliary iris-diaphragm occupies a position between the two collecting-lenses, and is controlling the diameter of the parallel beam. The image of the radiant as seen at the back of the sub-stage of the microscope is much smaller; but, as with medium-power objectives the aperture of the sub-stage condenser is of necessity less, or at least its effective working aperture is less, the projected image is sufficiently large to fill the field and does so without any reduction of aperture. In this case again the image of the closed auxiliary iris may be focussed in the field of view.

Reference to Fig. 36 *c* will show the arrangement for low powers. It does not differ in principle from the preceding arrangement, except that the lens No. III, of shorter focus than No. II, is substituted. The lens No. III is in fact similar to No. I, but, instead of having its plane side towards the illuminant, is placed with its convex side in that direction, so that it throws a small image of the source of light on to the sub-stage iris-diaphragm. The same conditions are here again to be complied with as in the preceding example, and the image of the auxiliary iris is once more focussed in the field of view.

It is assumed in the latter case that the sub-stage condenser is one of large aperture, and will be used for higher power work, but has had its top combination removed. The working aperture of the condenser is, of course, again reduced ; but this is not a matter of moment, as the objectives to be used in conjunction with it are of proportionately smaller aperture. These arrangements are to some extent influenced by the illuminant in use. For instance, the combination of Nos. I and III collecting-lenses, if used in conjunction with a Welsbach inverted incandescent light, or any illuminant of large area, will answer perfectly for medium as well as for low powers, so that the conditions may be altered somewhat according to the size of the illuminant. Again, the arrangement of the auxiliary collecting-lenses is to some extent dependent on the sub-stage condenser, as on its focal length to a great extent depends the size of the image of the illuminant that is projected into the field of view. Variation within some limits is therefore possible, depending on the particular sub-stage condenser.

An improved type of collecting-lens is now made by Messrs. Zeiss which supersedes the three lenses described. This lens is very thoroughly corrected for spherical aberrations, the correction having been obtained by the use of aspherical surfaces. It is a single lens fitted with an iris diaphragm, the image of which can be projected to any desired position. Its aperture is large enough for any purpose and it can of course be reduced as required. The main advantage of such a lens is that it is suitable for any purpose where a small light-source, such as an electric arc or Pointolite lamp, is used. Owing to its correction the quantity of light concentrated on any desired area is about four times as great as with an uncorrected lens of the same focal length. In other respects little is gained by using a lens of this character ; the resulting photograph is not of necessity any better than can be obtained with a simple lens. Although the set of three collecting-lenses already described has been superseded, they are in fact a useful set for many purposes. Further, the method of using them may be applied to any plano-convex lenses of suitable focal lengths. Such may be bought for a few shillings from any optician and can be supported in any convenient way in an improvised mount. Their focal lengths may approximately be as follows :

No. 1 about 7 inches focus. No. 2 about 12 inches. No. 3 about 6 inches. Such will be found to cover practically all requirements and their use will form a valuable exercise in practical optics.

Whatever auxiliary collecting-lenses are adopted, it is important to see that they are so adjusted that no reduction in the available working aperture of the sub-stage condenser results from their use. This may be determined by first obtaining an image, in the manner already described, without any optical system intervening between the source of light and the microscope. Then remove the ocular and see, by looking at the back lens of the objective in the manner also previously described, what proportion of the objective-aperture actually is being used. Now arrange the auxiliary lenses in the manner required; there should then be no decrease in the working aperture as seen by looking at the back lens of the objective. Whatever auxiliary lens is used, it must also be seen that the back lens of the sub-stage condenser is, at least for high powers, perfectly filled with light.

The proper use of these auxiliary collecting-lenses is a matter of considerable moment, and those who are not familiar with them are strongly advised to study, by the method already set out, the effect of altering their position, by observing a projected image on a white reflecting-surface. More information will be gained by some preliminary experimental work of this character than by reading pages of description. The worker should set up his various parts in the manner described and note the results, and should then vary the relative positions of these parts and see exactly what happens. It is as important to be able to recognise any error and to determine its cause, as it is to set up the apparatus with precision and in proper working adjustment. It is necessary to see that the iris-diaphragm, or any stop used as its substitute forming a part of the auxiliary collecting system, does not affect the working aperture of the sub-stage condenser or the objective, assuming, that is, that the beam is sufficiently large to fill the sub-stage condenser with light. This iris-diaphragm should only control the effective size of the image of the illuminant, and therefore of the field of view. In all cases where this image of the diaphragm is the focussed image, it is advisable to close it to such an extent that its image just encroaches on the field of view. By this means any extraneous stray reflections are considerably reduced, and scattered light from portions of the object not in the field do not have a disturbing effect. By attending carefully to this point the purity of the resulting image is appreciably increased.

In the cases already described where one collecting-lens is placed near the source of light, and is allowed to act as a collimator, throwing a parallel beam on to a second condensing-lens, by placing a piece of white paper in the path of the rays it can be observed whether a parallel beam is being projected in the right way. The illuminated area should be quite even, with no darkened areas or bright spots in any part of it. Practically, of course, it is impossible to obtain a perfectly parallel beam of light, but a sufficient approximation to this may easily be secured.

To summarise, when using a single collecting-lens, of whatever type, the image of the lens-aperture must be projected in the plane of the object, or alternatively the aperture of a diaphragm placed close to the lens may be so focussed. With a compound system of collecting-lenses a diaphragm may be placed in a position where a parallel beam is being projected and an image of the diaphragm-aperture thrown on to the object by the sub-stage condenser. In either case steps must be taken at each stage to ascertain that the full working aperture of the sub-stage condenser is being utilised.

Any collecting-lens must be placed exactly in line with the optical axis of the microscope and with its face at right angles to the optical axis. The latter adjustment is best secured by observing the faint image that is reflected from each lens surface. The reflected image should return in the same direction as the direct beam, and can usually be seen as a more or less central disc of light on any diaphragm in the path of the rays.

The photography of large sections or similar transparent objects by transmitted light is in some respects the most difficult branch of photo-micrography. Even illumination, on which success depends, can most easily be secured in the manner to be described. A microscope is not necessary, the objective used, whether of a short-focus photographic or Planar type, may be mounted on the front of the camera. Illumination is effected by means of a lens, the diameter of which must be greater than that of the object. The principle is the same as the projection of lantern slides, and an ordinary lantern or enlarger condenser is quite suitable. Reference to Fig. 82 will show the arrangement. A is the source of light, B the condensing lens combination, and C the object supported in a suitable carrier close to this lens, as a lantern slide is disposed for projection. The photographic lens D is placed approximately at the position where an image of the radiant is formed by the condensing-lens, but this position is in large part dependent on the enlargement required. E is the focussing-screen on which the image of the object is projected and is the position occupied by the photographic plate. Once the object is focussed, any adjustment that may still be needed to secure even illumination must be made by altering the distance between illuminant and condensing-lens.

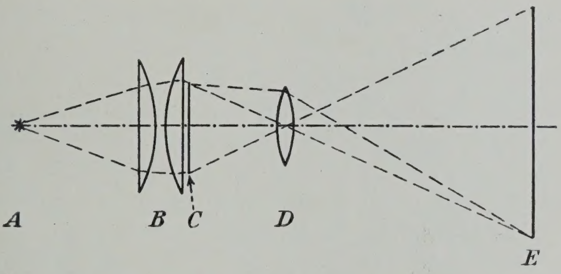


Fig. 82.

Very large sections can be photographed by simply putting them in contact with a slow photographic plate and exposing them to a small light-source placed at a considerable distance to reduce parallax. A suitable colour-screen can be placed near the light. No condensing-lens or any other optical device is needed; the exposure is made through the slide with the photographic plate in contact with the cover-glass. This provides a negative in natural size, and enlargement or reduction can be carried out by ordinary photographic methods. This simple arrangement overcomes the considerable difficulty often experienced in evenly illuminating large preparations, in which the magnification must of necessity be low, or in which to obtain lantern slides for projection the photograph must be smaller than the original object.

Dark-ground Illumination.—There are many methods of adjusting dark-ground illuminators, but the following has the merit of simplicity, and if carried out with reasonable care is sufficiently correct for any purpose. It is also an efficient means of adjusting any sub-stage apparatus. The illuminant is first set up in the manner already described, with its collecting-lens in correct relation to the mirror of the microscope. No optical appliance is put into the microscope itself with the exception of a low-power ocular. The light is then directed up the tube by adjustment of the mirror so that the eye-piece is evenly and completely filled with light. That this has been accomplished may be ascertained in one of two ways. If the observer places his eye exactly over the ocular and at a distance of from eight to ten inches from it, he will see, if the adjustment is correct, a perfectly even disc of light. A more exact method if the illuminant is sufficiently powerful, is to project a disc of light on to the ceiling; if that is impracticable, on to a piece of thin paper held three inches or more above the microscope. Fig. 83 shows the actual operation of adjusting the mirror. It is assumed that the light and collecting-lens are in perfect alignment with the centre of the mirror. Incidentally it is important to determine that the mirror is mounted centrally to the body-tube of the microscope, a point to which

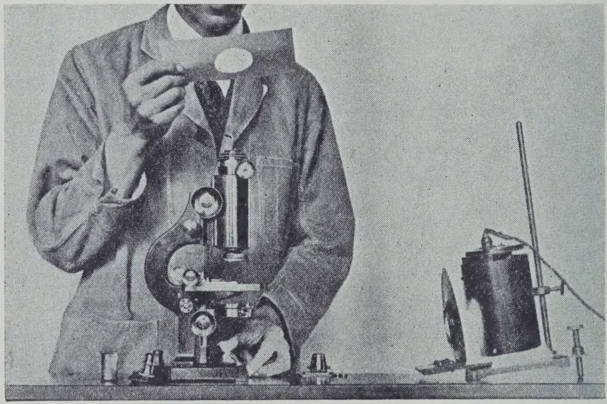


Fig. 83.

makers devote too little attention. The left hand may conveniently grasp the mirror, adjusting it on each axis in turn. It is possible to make the necessary adjustments by observing the image of the light reflected from the mirror through a dummy eye-piece with pin-hole in the cap, but this method is not so satisfactory. If there is any lack of centration, due to the beam not being projected along the optic axis of the microscope or to the image of the source of light not falling in the centre of the mirror, it can at once be ascertained and corrected by manipulation of the mirror or alteration of the position of the light. This adjustment having been effected, the next step is to slide the dark-ground illuminator into the sub-stage, taking care not to move the microscope nor to disturb the mirror. Place a low-power objective on the microscope and an object on the stage, having previously put a large drop of cedar-wood oil or glycerine and water on the top of the dark-ground illuminator; carefully rack up the latter so that the under-side of the object slide is immersed and is nearly in contact with the top of the dark-ground illuminator. Now get the object into view by focussing the objective. On observation it will be seen that the object is illuminated by a disc of light. By means of the sub-stage rackwork, the illuminator should be moved up or down until the smallest possible area of the object in the field of view is illuminated. Probably it will now be found that the illuminated disc is not central, and this must be corrected by the centring screws of the sub-stage, or by a centring fitting on the nose-piece of the microscope, if the latter alternative is adopted. Under no circumstances should the relation of the light to the microscope be interfered with after the preliminary adjustment described has been carried out. Any correction necessary to ensure centration must be effected entirely by the centring screws, either of the sub-stage or the nose-piece. The higher-power dry or immersion objective is now substituted for the low-power one and the object again focussed. It may be found that some want of centration is apparent, due to the optic axes of the objectives not being coincident. This again may be corrected by the centring screws. A higher-power ocular may now be used, and the apparatus should be in perfect adjustment. It should be remembered that when using a dry lens any alteration of tube-length necessary to correct for different thicknesses of cover-glass must be effected, and this should be the final operation.

In most cases the troubles that arise in using dark-ground illuminators are due to the imperfect adjustment of the light and collecting-lens in relation to the mirror. To overcome this difficulty the method in use at the National Institute for Medical Research is to remove the mirror from the microscope altogether. The light is placed low down on the bench and a truly horizontal beam is thrown by a collecting-lens on to a right-angle prism. This is mounted as shown in Plate VI, Figs. 1 to 3, so that it can be adjusted in height or if necessary slightly tilted to the axis of the beam. The plate carrying the prism has some freedom to slide about on the supporting block but is held in any desired position by a spring at the back. Any necessary change in tilt is effected by means of three screws which come through from the back of the supporting plate and move the metal disc carrying the prism. No doubt other methods can be devised for suitably supporting a prism for this purpose, but the essential point is that a horizontal incident beam shall be reflected by the prism so that it emerges truly vertical. This having been arranged for, it is only necessary to place the microscope over the prism and move it about until the light image is central. In our own practice the fitting is permanently screwed down to the work-bench, together with the light-source and collecting-lens.

It is well worth while to procure a simple micrometer gauge and to calibrate both cover-glass and slide, thus the trouble incidental to correction by means of alteration of tube-length is in part avoided. Such can be obtained from any optician, but a thoroughly satisfactory substitute is that made for engineering purposes and sold by most tool dealers. These read to 1/100th of a millimetre with accuracy, and are preferable to the cheap form of gauge with a long lever arm which is sold for optical purposes, and which is very easily put out of adjustment should the arm be knocked or bent. Thickness of slide is important and is determined by the focal length of the illuminator used. In most cases makers indicate this either on or with the appliance. With the Beck dark-ground illuminator of high aperture (N.A. 1·2 to 1·27) the best results are obtained by mounting the object between two cover-glasses, as, when the necessary

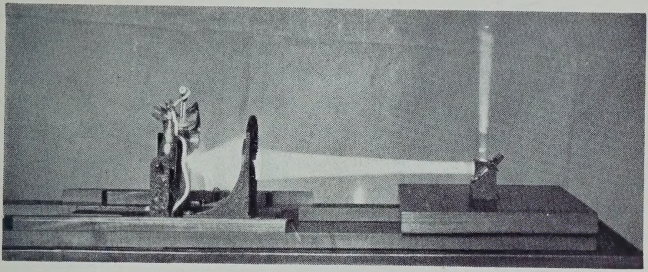


FIG. 1

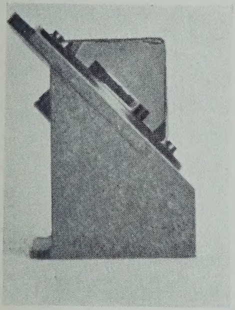


FIG. 2

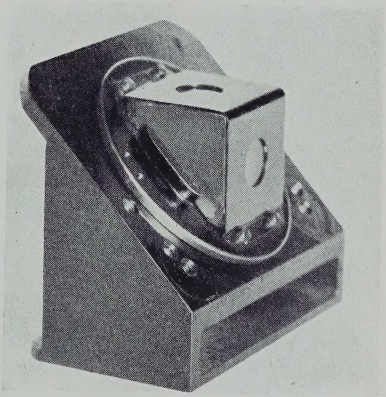


FIG. 3

Adjustment of Light and Collecting-Lens in relation to the Microscope.

conditions are at their best, the working distance of the condenser is very short.

Absolute cleanliness is one of the main conditions for successful manipulation. The cover-glasses and slides must be carefully cleaned by boiling them in a mixture of potassium bichromate and sulphuric acid, 10 per cent. of each in water. After boiling they are thoroughly washed in water to completely remove the acid mixture and stored in absolute alcohol in a suitable stoppered bottle or jar. To prepare a cover-glass for use one is removed with forceps from the alcohol and carefully rubbed between two selvyt-covered pads previously prepared by moistening with alcohol to which a small quantity of the finest rouge has been added. The pads consist of a pair of circular wooden blocks, about three inches across, over which the selvyt is stretched, with several layers of filter or blotting-paper between the wood and the selvyt. This polishing process removes all surface dirt, but it leaves a small deposit of rouge behind. This can be removed by a final rinse in absolute alcohol, the cover-glass while held by its edges being wiped dry with a perfectly clean handkerchief or a piece of Japanese lens paper. Slides may be treated in the same way, but the rouge polishing is usually unnecessary. Both objectives and oculars should be kept free from dust. If operations are carried out in a room where dust is prevalent, it is advisable if a dry lens is used, to occasionally dust the cover-glass with a small camel-hair brush.

The immersion fluid used with any illuminator must be free from air-bubbles. A method of securing this is to place a large drop of cedar-wood oil (or glycerine and water) on the top surface of the illuminator. Also place a small drop of the immersion fluid on the *under*-side of the object slide. See that the illuminator is down far enough to avoid contact of the drops when the slide is placed on the stage. Now carefully raise the illuminator until the two drops touch. Owing to the convexity of the contact surfaces of these drops, there is no tendency for air to be enclosed, and the result is that air-free immersion is secured.

CHAPTER VIII

GENERAL PRELIMINARY PREPARATIONS

Vibration and its Prevention.—Apart from the acquisition of suitable apparatus, due consideration should be given to other points affecting the final results. Possibly the chief among these is the situation of the room or apartment in which the work is to be carried out. It has already been mentioned how greatly vibration of the apparatus is to be feared, especially when taking photographs with high powers. It is obvious that such work is much influenced by the situation of the apartment in which the apparatus is fixed. Assuming that the work is being performed in an ordinary house, the best position for the room is in the basement. An additional advantage of having a basement apartment is that it is less subject to changes of temperature than a room situated at the top of the house. In the latter the change of temperature even between night and day is considerable at any time of the year, but in the former situation it will be found that the range is small even between the greatest heat of summer and the low temperatures of winter. Certainly a room with a northern aspect would be better suited than any other.

It is likely, however, that the majority of workers will have this point determined for them, and may have to use a room which is not required for other purposes. It is therefore necessary to point out some of the methods available for overcoming the troubles due to vibration. It is by no means certain that any apparatus is better for being firmly fastened down to the floor, unless that floor is of particularly solid construction. When an ordinary floor is the only means of support, some method of reducing the transmission of vibration may be necessary.

The simplest and most easily applied is to place under the feet of the apparatus three- or four-inch cubes of solid indiarubber. This reduces the smaller tremors, and effectually prevents any impulse short of a definite shock from being transmitted. A more drastic although somewhat more troublesome method, is to sling the whole arrangement on stout cords. The illustration (Fig. 84) shows a method of carrying a small apparatus on this principle. In this case the camera and microscope are supported on one board, which is slung between two metal uprights, themselves standing on an ordinary table. This arrangement is very efficient indeed where work has to be done in an ordinary room.

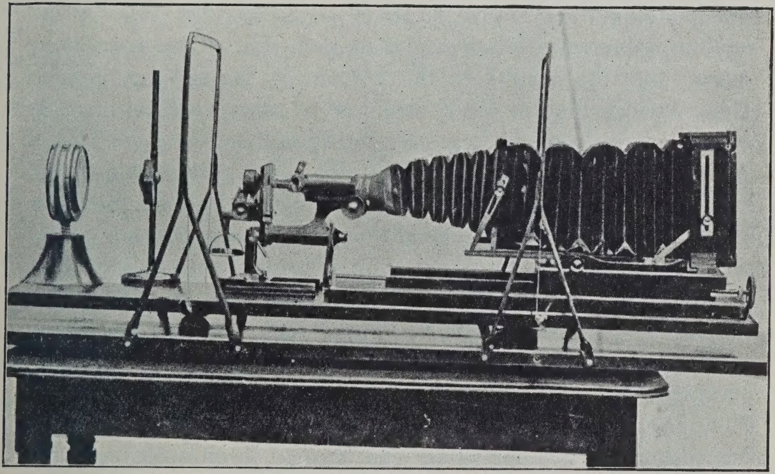


Fig. 84.—Method of supporting Camera to minimise Vibration.

If the camera is of a larger type, it will hardly be possible so to suspend it. In most cases where the weight is considerable it is better, if possible, to let the cords be hung from beams in the ceiling; much better isolation from movements of the floor, due to persons moving about in neighbouring apartments, is obtained.

In extreme cases thick india-rubber cords may be substituted for the ordinary cord.

Another method, which would appear to be more effective, but is more difficult to carry out, is to carry or hang the apparatus on strong steel springs. In this case it would not merely be necessary to let the weight of the whole appliance hang on the springs, but other springs should also be fixed so that they act in opposite directions to prevent the whole apparatus from acquiring a periodic oscillation or vibration of its own. It will therefore be essential to connect it by several springs to one support, so that any movement imparted to it is stopped at once whatever the direction it may take. One method of effecting this is to have a baseboard carrying the whole apparatus on two supporting-legs at each end, both of which are cross-braced to the under-side of the baseboard. A strong wooden supporting-frame is then so made that from three separate points at each end, and at each side of the apparatus, springs may be carried to the bottom of the supporting-legs. These springs require to be strong steel ones, such as may be purchased ready made from any good ironmongery store. It is only possible to indicate in general terms how this arrangement can be carried out, as details must be determined by the form of the apparatus and the conditions under which it has to be set up. But it will be found that, if properly made with due regard to the mechanical principles involved, some such spring support is perfectly efficient. The recent large photo-micrographic apparatus designed both by Leitz and Zeiss is supported on springs. Each has characteristics of its own, but there is no doubt as to the efficiency of the method of spring suspension. Either apparatus can be used in an ordinary room or laboratory, and the isolation from extraneous vibrations is practically complete. In some buildings with strong thick walls, by supporting the apparatus on heavy iron brackets cemented into the wall it is possible to avoid vibration. Such a method is commonly adopted in places where machinery is in operation and has proved effective.

When a light source of high intensity is used, such as an electric arc, vibration is less likely to be troublesome as the exposure is short. With less intense illuminants, where exposures may extend to several minutes, the danger is much increased. In either case a stable support for the whole apparatus reduces the risk.

It is not easy to overrate the disastrous effect of vibration when work is being attempted in an ordinary laboratory or a house, and many a photograph over which great care has been expended in other respects, has been spoilt by the want of attention to this troublesome factor. It is advisable for the operator and any others working in the same room to stand still during the period of exposure.

Light-excluders between the Camera and Microscope.—The microscope and camera are for all essential purposes two separate pieces of apparatus, but a connection must be made between the eye-piece end of the microscope and the front of the camera by some arrangement that will effectually prevent the entrance of extraneous light. This may simply be effected by having a velvet or cloth tube, with an elastic band at either end, which slips over the microscope tube and over a short tube protruding from the front of the camera. While this is quite efficient, it has the disadvantage that in the actual connecting up of the microscope to the camera there is considerable danger of the adjustments of the instrument being altered. The almost universal method is to use a light-excluding device, as shown in Fig. 85. This has the advantage of simplicity, is cheap and easy to construct, and works well under all conditions. Further, there is no actual contact between the microscope tube and the front of the camera itself. Let AA represent the camera front, and BB an open tube extending from its centre. This tube BB may be used for receiving a carrier for a microscope objective of low power, or an ordinary photographic lens for photographing culture-tubes or similar objects. On the ocular end of the microscope body-tube D a double tube CC is placed; the inner and smaller tube just slides over the body-tube, while the outer tube is sufficiently large to have at

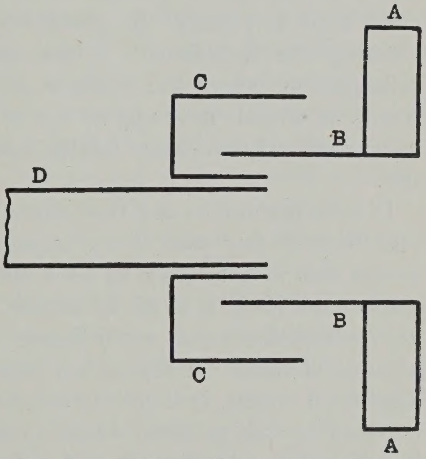


Fig. 85.—Appliance for excluding Light.

least one-eighth of an inch clearance outside the tube BB of the camera. When, therefore, the camera front is brought forward so that its tube lies between the double tube of the fitting CC, no extraneous light can enter between the microscope and camera. The whole of this appliance should be dead black to prevent the possibility of reflections from any of the exposed surfaces.

Diffused Light.—It is advisable to protect the whole of the apparatus from diffused light, whether from the illuminant or from any extraneous source. The illuminant should therefore be enclosed in a suitable casing with an opening, other than any necessary for the purpose of ventilation, only in the direction of the optical system. Some workers also advise that the whole of the optical system should be covered with a black-cloth hood to prevent the possibility of stray or diffused light entering the apparatus. While this may be an excellent arrangement where the work is carried on in a well-lighted room, it would not appear to be necessary in any special apartment where other bright sources of light are not in use.

It is important to see that there are no disturbing internal reflections in any part of the apparatus. In well-made microscopes this is not likely to be a source of trouble, but inside the camera itself it is by no means unusual to find that there are some surfaces that are diffusing or reflecting a considerable amount of light. If this arises from the apparatus not being dead black inside, it should be immediately rectified. This can be done by taking some dilute shellac varnish and adding to it sufficient fine lamp-black to form a mixture of a creamy consistency. This should be rubbed over the woodwork, and will effectually blacken any reflecting parts. If the inside of the bellows of the camera is not dead black, it is best to make a mixture of lamp-black and ordinary flour paste, and apply this where required.

It is a good plan to interpose dead-black metal diaphragms with suitable openings between the camera front and the ground glass focussing-screen. Many makers supply these thin metal plates with openings of various sizes which fit into the back of the camera, immediately in front of the position taken by the dark-slide. It is advisable when photographing to use a diaphragm with as small an opening as possible. Useless portions of the image should not be allowed to fall on the photographic plate, since they diffuse unnecessary light inside the camera body. A metal diaphragm may usefully be interposed about midway between the camera front and the photographic plate. It is a wise precaution to instal the apparatus in a room in which the lighting is subdued, and as far as possible to extinguish any ordinary lights.

Heat from Illuminant.—With most high-power illuminants particularly the electric arc, some method of absorbing the radiant heat is necessary. Otherwise expansion of the components is sure to take place, and sufficient dislocation to alter the focus will ensue during a long exposure, with the result that the image is not well defined. A further and more serious probability is that damage may be caused to the microscopical preparation and even to the objective. This may be prevented by placing a water-bath in a position as near as convenient to the source of light. Such an appliance is sold ready made by most opticians, but one may be constructed in the manner described for making cells to contain coloured fluids; this is referred to in a later chapter on that subject. The cell should be filled with cold well-boiled distilled water, to ensure that all the air is driven off; otherwise the heat of the radiant will cause air-bubbles to appear on the walls of the containing-vessel. It is sometimes advised that the absorbing fluid should be a saturated solution of alum instead of water only, but this would not appear to increase the heat-absorbing power. When a yellow screen is used, a weak solution of perchloride of iron may be substituted for plain water, and will much more effectively absorb the heat rays. The greater the heat emitted by the radiant, the larger the water-bath should be, so that there is sufficient water to prevent any considerable rise of temperature and consequent injury to the objectives during a long exposure. In any case the cell should be at least one inch in thickness. The most efficient heat-absorbing solution at present available is as follows:

Ferrous ammonium sulphate .	10 grammes
Distilled water	100 c.cs.

The solution is made with cold water and if it appears turbid dilute sulphuric acid should be added in small quantities until it clears. The solution does not keep well, and should be discarded as soon as it turns at all yellow. When fresh it is nearly colourless and has little effect on the actinic value of the light. A cell about half an inch in thickness is sufficient in addition to the ordinary water-bath except for high-power arc lamps, but the latter are now rarely used.

Diaphragms.—It is convenient to interpose between the source of light and the microscope one or two diaphragms, either of the iris pattern or of the type in which a series of metal plates with openings of various sizes can be inserted at will. It is usual to have one of these as close to the illuminant as possible. In the case of illuminants of rather large area—such as the mercury-vapour lamp—one may be placed almost in contact with the tube of the lamp itself. It is well that any part of the illuminant not in actual use should be effectually screened off. When collecting-lenses—such as the Zeiss-Kohler lenses already described—are being used, a diaphragm may also be interposed between the two lenses. With any system of illumination it is advisable for the illuminating beam to be contracted to such a size that no portion of the beam, other than that actually in use, is allowed to reach the microscope. In any case where one or two diaphragms are used the position should be chosen so that any extraneous or unused light is effectually screened off. The iris-pattern diaphragms are obviously the most convenient, but the cheaper metal plates with suitable sized openings are perfectly efficient.

Size of Camera.—This is a point of no particular importance, but when a camera is being obtained especially for the purpose, it is advisable that its size should not be greater than is really necessary. In the large majority of cases what is known as a quarter-plate camera is sufficient, as in any other than low-power work the area of good definition will rarely exceed that of a circle of three inches; consequently, the plate of $3\frac{1}{4}'' \times 4\frac{1}{4}''$ (quarter-plate) will suffice.

When using Planar or similar low-power lenses, however, the area of good definition is considerably larger, so that it may be more convenient to adopt a half-plate size, $6\frac{1}{2}'' \times 4\frac{3}{4}''$. This will entail the use of plate-carriers when using plates of smaller size. It is important to see that these plate-carriers do not interfere with the register of the plate in any way; that is, either a small or large plate when put into the dark-slide should be in exact register with the focussing-screen of the camera. In general, these conditions are complied with by camera-makers, but it is advisable to make sure that there is no difference in their position before work is actually attempted.

This may be done roughly by measuring the distance of the focussing-screen, or of the sensitive surface of the photographic plate, from any fixed position in the camera itself. A more efficient method is an optical one. An ordinary photographic lens of wide aperture should be put on the camera front and some sharply defined object—such as a window frame, or even better a sheet of printed matter—carefully focussed on the ground-glass focussing-screen. The camera dark-slide should then be inserted, and, assuming that it is a double back which will allow of one-half of the slide being turned back and the plate in position being exposed to view, a piece of ground-glass may be put into the dark-slide in the position usually occupied by the sensitive plate. The image is then observed, and should, under these conditions, be equally well defined when viewed on either the proper focussing-screen or on the ground-glass inserted in the dark-slide carrier.

It must be admitted, however, that in photo-micrography such perfect registration does not affect the final result to the same extent as in ordinary photography. The angle of the beam is usually so narrow—that is, the rays approximate so closely to parallel rays—that a small difference in position between the dark-slide and the focussing-screen does not have so great an effect. Since, however, the camera may be used for varying classes of work, it is a point well worth attending to; so that whatever may be attempted no depreciation of the result need be anticipated from such a source of error.

If greater exactness in focussing is required, a piece of plain glass may be substituted for the ground-glass, and the image focussed by means of a good focussing-magnifier. This method is both easier and more efficient than taking a succession of photographs to ascertain the correctness of register of the dark-slide or its carriers, as any possible aberration, due to the lens with which the test is made, is then entirely eliminated.

One point of considerable importance, affecting both the dark-slide and the focussing-screen, is that they should easily drop into their position at the back of the camera without jar or dislocation of any part of the apparatus. The method of inserting either the one or the other in a long groove should therefore be avoided; it is sufficient that they drop in at the back either in a very short groove, or even better, are merely held in position with a simple form of catch. It is also important to see that the shutter of the dark-slide moves very easily and sweetly when it is being withdrawn; nothing is more disconcerting than for an unforeseen jar to disturb any part of the apparatus as the result of removing the focussing-screens, inserting the dark-slide, or withdrawing the shutter, and too much attention can hardly be given to these points to ensure that all the movements are perfectly smooth and can be made with the least possible risk of disturbance. As the room in which the work is being carried out should be partially or completely darkened, there is very little risk of extraneous light entering the camera at this point.

Focussing-screens.—The camera should be provided with two focussing-screens: one of ground-glass and the other of plain glass—the latter patent plate to ensure that there is no inequality of surface. The ground-glass screen or its substitute should not be extremely fine, for this is a point that may be carried too far. If a screen is too translucent, such a large amount of light is lost by direct transmission that the image loses in brilliancy. On the other hand, with too coarse a screen the grain becomes so painfully obvious when focussing that the fine details of the image are lost. It must be pointed out that a great deal of the focussing is best carried out on the ground-glass screen, so that careful attention to this point is advisable.

There are many substitutes for ground-glass, but although their grain is fine they nearly all have the disadvantage that the screen itself is too translucent, thus resulting in considerable loss of light. In practice it will be found that ground-glass a little coarser than the finest that can be obtained is the most efficient, and, if used in conjunction with suitable focussing-glasses to be described, there are many cases where exact focussing can be effected without recourse to any other screen. When fine detail or a particular portion of an object is to be focussed, recourse must be had to the plain-glass screen. This should have two light scratches ruled by a diamond in the direction of the diagonals of the plate crossing at its centre, and these scratches should be carefully focussed with the focussing eye-piece before focussing the microscopic image.

An alternative method, and one which may be adopted where a ground-glass screen only is provided with the camera, is to cement on to the centre with Canada balsam an ordinary microscopic cover-glass. The Canada balsam has the effect of making this small area perfectly clear, effectually eliminating the ground-surface, and the image can therefore at the same time be observed over the whole remaining area of the screen. For fine focussing purposes this central area will often be found sufficient, but it is open to the objection that the clear area is very limited, and consequently, should the part of the image to be focussed lie at any spot other than the centre, it cannot be observed satisfactorily.

A substitute for the ground-glass surface may be made by coating a piece of patent or thin plate-glass with a matt varnish made as follows:

Sandarac	1 gramme
Mastic	0·25 gramme
Ether	6 c.cs.
Benzole	from 2 to 4 c.cs.

The finer the required surface, the greater the degree of dilution of this solution—that is, more benzole should be added so that a finer film is deposited on the glass. This will give a very fine focussing-screen, but it is open to the objection that the matt surface is liable to abrasion, and in course of work, where a screen is handled a good deal, this becomes a serious disadvantage.

The finest focussing-screens are those made by chemical means. Take an ordinary photographic plate, preferably an extra-rapid one, and without any preliminary exposure to light, immerse it in the dark in any non-staining developer free from potassium or ammonium bromide. The plate may be left in this for from five to twenty minutes. The longer periods result in a fine and translucent screen, and the shorter periods in a screen that is equally fine and free from granularity, but less translucent. At the end of this period of development, the plate is washed and fixed in the ordinary way. It is then immersed in a solution of iodine in potassium iodide; the exact strength of this solution is not important. The plate may be left in this solution for some few minutes until completely iodised, then again washed and immersed in dilute ammonia, and finally washed and put to dry. This screen is useful when the plain-glass screen does not fulfil its purpose. If used in conjunction with a focussing-ocular, the grain is so fine that there is no evidence of it, even if the ocular is a fairly high-power one. The difficulty of accommodation that occurs in some cases when using a plain-glass screen, is entirely absent when fine-grain screens are used, giving a concrete image.

The ground-glass screen may be rubbed over with a small quantity of oil in cases where it is too coarse, or where a feeble source of light is used and it is not sufficiently translucent. It need hardly be mentioned that the ground-surface of the ground-glass screen, or in the case of the plain screen, the surface which has the diamond scratches on it, should be towards the light.

A useful accessory is an opaque white screen, placed in a suitable position so that the microscopic image can be projected on to it, and this image observed to see if the centration or other adjustments of the illuminant or optical system are correct. With an apparatus of the type in which the microscope and accessories swing out of the optical centre, the image can easily be projected on to such a white screen placed at either side of the camera back.

If reference be made to Plate IV, Fig. 3, one method is shown of arranging such an opaque screen. It consists essentially of a piece of plain board which is hinged to the side of the frame carrying the focussing-screen of the camera, and on the side towards the microscope a piece of plain white paper or cardboard is fastened. The white surface is in alignment with the focussing-screen of the camera, but as it is hinged on a vertical axis the position may be altered when the microscope is swung out. Obviously this screen must be at a different inclination to the camera according to the length of camera in use, the hinges allow of variation of the inclination. The image may be approximately projected on to the opaque screen in the position in relation to the microscope that it would occupy on the ground-glass focussing-screen. With this arrangement it is possible to make all the adjustments—centring of the sub-stage of the microscope, centring of the object, focussing of the projection-ocular diaphragm, approximate focussing of the object itself—and to observe finally if all the adjustments are correct, while standing in a position near the microscope. This renders the work of adjustment very much easier, and any correction can be made without having to move backwards and forwards from the ground-glass screen to the microscope, while the effect of any alteration is immediately seen. On this, as well as on the ground-glass screen in the camera, diagonal lines should be drawn so that their point of intersection indicates the position that should be occupied by the centre of the object. It is also useful to describe a circle indicating the largest image that may be photographed on the sensitive plate to be used. In the case of a quarter-plate this circle could not be more than three inches in diameter.

Focussing Eye-pieces or Magnifiers.—When using the plain-glass focussing-screen an achromatic eye-piece, usually of the Ramsden type, must be employed. There are many types of these on the market, and the one illustrated (Fig. 86) is typical. It must be adjusted so that when placed on the plain focussing-screen the diamond lines on the far side of the screen are exactly in focus. This must be done for each individual user ; but, having once been correctly set it remains always ready for use. The adjustment is effected by screwing the cell carrying the optical portion in or out by means of the milled head shown, until the image of the diamond line or of the ground-glass surface is sharply focussed. When this operation has been carried out, any alteration of

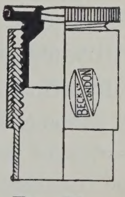


Fig. 86.—Focussing Glass. (R. & J. Beck, Ltd.)

the position of the lens system is prevented by firmly screwing down a clamping-ring.

In general, these focussing eye-pieces are of too high power for photo-micrographic work, as the image—at least in high-power work—is not one that will stand great amplification. Messrs. J. Swift & Son make a focusser which answers better than any of those on the market. It is a well-achromatised system but of very low power, so that the image is magnified less than two diameters. In effect it takes up the image at the focal plane, and transmits it to the eye with but slight alteration. The only objection is that the field is small, but in the majority of cases this is of no moment. Such a focussing system is fitted into a long mount, and owing to this it can only be used at or near the centre of the focussing-screen. If it is away from the centre to any considerable extent, the image is cut off by the sides of the mount. The great advantage of having such a low-power eye-piece for focussing is that the image is practically unaltered, and it is easier therefore to focus images formed by high powers.

Some workers have advised the use of ordinary spectacle lenses for focussing, and this would appear to be quite practicable where only a ground-glass screen is used. Better still is it to use a pair of spectacle lenses mounted to form a binocular focussing-glass. This type is made in a convenient form by Messrs. Newman & Guardia, and is supplied by them for use in their reflex cameras. The binocular, as supplied, should be mounted on a simple tube or box, so that the distance of the eye-pieces from the focussing-screen is the same as in the reflex camera for which they are designed. Messrs. Charles Baker also supply binocular magnifiers of various focal lengths to suit individual requirements.

Position of Microscope in Relation to Camera.—A point of primary importance, although it is not often referred to, is to ensure that the optical system is in perfect alignment with the camera, both vertically and horizontally. This is not always the case, even though the apparatus has been constructed especially for the purpose by a scientific instrument maker. If the microscope and other optical parts swing out of centre, as already described, they may not return exactly to a position in which the optical axis is directly in alignment with the centre of the camera. Again, the microscope itself when brought into a supposed horizontal position may not be truly horizontal. Any want of truth in either of these directions may be determined by observing an image on the focussing-screen of the camera. As a test-object for this purpose nothing is better than an ordinary glass object-slide on which two fine lines have been ruled at the centre of the slip exactly at right angles to one another. They are focussed in the microscope with a medium-power objective, and the image projected on to the ground-glass screen so that the point of intersection of the diamond lines is truly in the centre of the screen of the camera. On moving the back of the camera carrying the focussing-screen backwards and forwards, from its greatest to its shortest length, no alteration in the position of the image should be observed. This condition is rarely exactly complied with, and more often than not it will be found that some alteration, either laterally or vertically, occurs in the position of the image. Careful observation will at once show in which direction there is any deviation from correct alignment, and steps must be taken to rectify this. It is evident that the direction of the movement of the object on the screen will be opposite to the direction of error of alignment. Any such error may sometimes be traced to the microscope itself, since in placing it in position on the base-board it is usually assumed that the points of the feet are true, an assumption that is often not justified. Vertical deviation may also be traced to the same source, as in even the best microscopes it is not unusual to find that the optical axis of the instrument is not truly horizontal. These errors are most easily detected by watching the projected image in the manner described, since this forms a far more exact test than any method of direct measurement.

Methods of Exposing.—In many ready-made cameras an exposing-shutter which is actuated by mechanical or other means is fixed at the front. The disadvantage of all such arrangements is the danger of vibration being imparted to the apparatus; and as such vibration occurs when an exposure is being started, the result may be harmful.

It will be found more satisfactory to have some simple arrangement for effecting the exposure. The simplest of all is to interpose a piece of black cardboard between the microscope and the nearest auxiliary collecting-lens. This may be supported as near to the microscope as possible, but yet not touching any part of the instrument, and may be removed at the commencement of the exposure and replaced at the end. It will not be found, at least when working in a room which is even partially darkened, that enough reflected light enters the camera to have any effect on the photographic plate. When the work is carried on in a well-lighted room some arrangement inside the camera may become necessary. The simplest is to have a thin metal flap or plate which should be as light as possible, carried inside the camera front on a hinge or thin spindle. The plate should be rigidly connected to the spindle, and the latter may project through the front of the camera on either side, with a milled head which may be actuated at either or both ends from outside the camera front. On rotating the milled head the plate either completely closes the aperture in the camera front, or moves downwards and lies in the bottom of the camera, leaving therefore a clear aperture where the microscope and camera join.

Alternatively any of the mechanical photographic shutters may be used, and may be fixed in the front of the camera. But unless one of the modern between-lens shutters of good construction is fitted—and this is really an unnecessary and expensive luxury—it will be found that some vibration is bound to take place.

It should be mentioned that when using very low powers the blackened card or its substitute may be interposed between the microscope objective and the object, thereby minimising to a considerable extent the chance of reflected light entering the apparatus. It is obvious that such reflections are more likely to occur with low-power lenses than with high-power ones, since the former transmit so much more light as they cover a larger area.

Various methods for effecting the exposure will suggest themselves to the worker, and any one may be adopted that does not result in disturbance of any part of the apparatus, and yet ensures that the sensitive plate is protected against stray light.

Focussing.—The various appliances for focussing the image have already been described, but there are one or two further points that may arise in the course of their use. It is of course necessary that any worker should first become conversant with the use of the fine-adjustment on the microscope under the ordinary conditions of visual work, and it is assumed that this elementary technique has already been mastered.

In photo-micrographic work the fine-adjustment usually is actuated by means of a long focussing-rod, which the observer can rotate at one end, and which is connected at the other end to the fine-adjustment milled head on the microscope itself. Any focussing arrangement adopted should act on the fine-adjustment with precision. The method should be such that the image only moves in and out of focus when the focussing-rod is rotated, and so that no lateral displacement of the image occurs. The rigid method of connection adopted in the Zeiss model, in which the motion is imparted to the fine-adjustment by means of a Hooke's joint, usually causes some slight lateral movement of the image when used with high powers, although it must be admitted that the actual focussing can be done with great exactitude. The movement of the image is perhaps only what might be anticipated; for the weight of the moving parts is such that there is considerable thrust, and it is only natural that this should become evident when the mechanism is actuated or the direction of movement reversed. Perhaps the most important point is that the fine-adjustment should stop precisely at any desired position, there should be no suggestion of continuation of movement beyond the position of correct focus. This not only applies to the use of mechanical controls in photo-micrography but should be remembered in connection with fine-adjustments in general.

In most high-power work it will be found that the plain-glass screen, used in conjunction with a focussing eye-piece, will be the most satisfactory; but it often happens with bacteriological or similar preparations that it is easier to do the fine focussing on the ground-glass screen. It must be remembered that these minute bodies are themselves either cylindrical or spherical, so that there is no absolutely sharp edge on which to focus. It therefore follows that a better idea of the actual image is conveyed when it is seen on a screen that gives a concrete image.

With high-power illuminants the image is sometimes too brilliant to focus with safety by means of a focussing-ocular on the plain-glass screen. In this case the sub-stage condenser-diaphragm must on no account be contracted; apart from the fact that the optical conditions are interfered with, there is always the risk of decentration of the sub-stage condenser if the diaphragm is interfered with at such a late stage of the operation. If there is too much glare, the light must be modified by placing neutral glass, or a piece of the colour supplied by some opticians for this purpose—a dark blue-green—between the microscope and the source of light. An effective glass is to be obtained from Messrs. Hetley of Soho Square, which conforms to the required conditions as it appears to reduce the quantity of light transmitted without altering its colour-value. This is a matter of considerable importance when colour-screens are in use; for, should a glass which has a power of selective absorption be used, it would disturb the arrangements already made and affect the resulting image. It is, of course, understood that any light-modifier of this description is removed before the actual exposure takes place. The most satisfactory light-modifier is semi-platinised glass, and such may be obtained from Messrs. J. Rheinberg & Co., London. The advantage of this glass is that absorption of light is almost equal throughout the whole visible spectrum. It is not difficult to make for microscopic work and a standard method for so doing will be found in the Appendix. Two or three pieces of such glass may be used together to obtain sufficient density, or if it is being coated by the user in the manner described successive coatings may be given until the desired density is obtained. A neutral-wedge light modifier is made by Messrs. R. & J. Beck, Ltd., by which any degree of light absorption may be obtained at will, but this is more suitable for visual work. Good neutral screens may also be had from Messrs. Kodak, Ltd.

The focussing should be carried out at the centre of the field —that is, with the centre of the field in the microscope projected exactly on the centre of the ground-glass focussing-screen. It may be that the particular point in an object to be shown does not lie exactly at the centre of the field, and in this case of course the above will have to be departed from. But in general it will be found more satisfactory to focus one central point in the object and allow the remainder to take its chance. This applies particularly when using apochromatic objectives since their field is small; it therefore becomes more important that the essential part of the image should be central rather than to attempt to focus any zone that lies away from the centre. Some recent forms of photo-micrographic camera have already been referred to and described in which a focussing device is embodied in the camera. The image is therefore focussed in the visual eye-piece and without any further adjustment the object is correctly focussed on the plate or film. This method needs no further description, it can only be used as part of the apparatus as supplied. In some cases the image seen in the ocular is not the same magnification as the resulting photograph and the method therefore has its limitations.

Magnification.—The magnification of microscopic objects is usually expressed in diameters, and not in terms describing superficial area. An object is said to be magnified a certain number of 'times'—this referring to the number of diameters that it is enlarged. The same general conditions apply as to any enlarged image projected by an ordinary lens—such as a photographic or a projection one. The magnification therefore of a projected image, if the superficial area is considered, varies to exactly the same degree as the intensity of illumination, i.e. as the square of the distance. In visual work magnification is estimated at a distance of ten inches from the eye-cap of the eye-piece, as this is the recognised distance for normal vision. Achromatic objectives are frequently described as of somewhat longer focus than they actually are, so that a one-inch objective is often something less than that, and may be as short as three-quarters of an inch. An objective of exactly one inch focal length would magnify ten times at a distance of ten inches from its upper principal focal plane. It is sufficiently accurate for all ordinary purposes to measure this distance from the upper lens of the objective, so that an image projected into the camera at a distance of ten inches from that point would give a magnification of ten times. If the camera-length is increased to double the above dimension, the magnification expressed in diameters is then doubled, and therefore becomes twenty times, and so on for any increase of distance. We therefore get for this distance of ten inches the following as the initial magnifications to be obtained with lenses of the indicated focal lengths :

A lens of	1	inch focus or	25	mm.	= ×	10
,,	$\frac{1}{2}$	,, ,,	12	,,	= ×	20
,,	$\frac{1}{4}$	,, ,,	6	,,	= ×	40
,,	$\frac{1}{8}$	,, ,,	3	,,	= ×	80
,,	$\frac{1}{12}$	,, ,,	2	,,	= ×	120

These are all on the assumption that the focal length of the lens is exactly that indicated ; but on actual test it rarely happens that this is found to be the case. It must be remembered that these figures represent the magnification under the conditions specified. When an ocular is used with an objective corrected for a short tube-length, say 160 mm. or 170 mm., the initial magnification is proportionally reduced. As an example the 2-mm. objective instead of having an initial magnification of × 120, would have $\frac{120}{1} \times \frac{160}{250} = 80$ approximately, when the tube-length is 160 mm.

In photo-micrographic work there are three factors that can influence magnification : the power of the objective, the power of the ocular, and the length of the camera. It is therefore possible within limits to obtain the same magnification with different objectives—and the method adopted to obtain any given magnification should be determined with reference to the object itself.

It has already been pointed out that the quality of penetration depends on the N.A. of the objective, and in general this N.A. is dependent on the focal length of the lens. In cases, therefore, where an object is of considerable thickness, and it is necessary to obtain the best possible compromise of several planes, it would be advisable to use a lens of the greatest focal length, that is of the lowest practicable magnifying power, and to obtain the necessary magnification either by using a higher power ocular, or by increasing the camera-length. No definite rules can be laid down in this matter, and it is only by careful consideration in relation to the object itself and after perhaps a fair amount of experience, that the best combination can be exactly determined. Apart from the magnification it is necessary for any objective to have sufficient numerical aperture for the required details in the object to be properly resolved.

Apochromatic objectives are usually more accurately described than achromatic ones, so that in estimating magnification solely in reference to the indicated focal length of the objective the margin of error is likely to be small. Their focal length is described in millimetres, and the chief ones are indicated in the foregoing table. When the image is projected with an apochromatic objective and no ocular, the initial magnification indicated is obtained at a distance of 160 mm. or 170 mm., or in the case of long-tube objectives 250 mm. from the objective end of the microscope body-tube. When a projection-ocular is added, the image, magnified 2, 4, or more times according to the ocular, is then obtained at a distance of 250 mm. measured from the ocular itself and not from the objective.

With projection-oculars it is fairly safe to assume that the magnification is in accordance with the number engraved on them, so that a × 2 or × 4 projection-ocular will magnify the image two or four times as indicated. But should the ordinary eye-piece be used, indicated by the letters A, B, C, and so on, or by some arbitrary number, the only way is to determine magnification by the projection of a micrometer ruling on to the ground-glass screen of the camera.

The influence of camera extension is very similar in effect to a change of ocular. If the ground-glass screen is ten inches from the eye lens of the ocular, we then obtain magnification in proportion to the focal length of the objective, multiplied by the number of times that the ocular magnifies. When the camera is extended beyond ten inches, the amplification is then directly proportional to the increase of camera extension. For instance, if we place an object on the stage with a one-inch objective and a No. 8 eye-piece, we shall find the object to be magnified approximately 80 times at ten inches' distance. But if we double the camera-length we should now get an object twice that size, equal to a magnification of 160 times.

It is convenient on any photo-micrographic apparatus to have a scale placed at the side of the camera showing the extension from the ocular. If an exact scale in inches or centimetres is not provided, a series of marks showing each ten inches of extension will be found useful. It is then only necessary to observe the length of camera taking into consideration the ocular and the objective, when by a simple arithmetical calculation the magnification obtained on the ground-glass screen can approximately be estimated. For most objects such an approximation is sufficient, and it is only when the exact magnification has to be determined that other methods need be resorted to.

If the estimation of magnification is not sufficiently accurate when arrived at by simply considering the focal length of the lens, the power of the ocular, and the camera extension, recourse must be had to the projection of the image of a micrometer ruling. This is a scale ruled on a cover-glass which is mounted on a 3″ × 1″ glass slip. The rulings are generally one-tenth and one-hundredth of a millimetre apart. It will be best perhaps to carry out the first estimate of magnification by means of a medium-power objective—say, one inch. Turn the microscope to the horizontal position, having the ruling on such a micrometer in focus, and project the image on to the ground-glass screen of the camera. The screen should be at a considerable distance, if the camera will admit of it—say, five feet from the front lens of the objective. If the camera extension will not admit of an image being projected to this distance, then it will be sufficient, if the source of light is fairly powerful, to throw the image on to a white screen or card at the required distance. We thus have a projected image at sixty inches' distance, and on reference to this image if we find, say, that the rulings, each of one-tenth of a millimetre apart, when projected on the ground-glass screen, have a distance between them of 6 mm., we shall know that the initial power of the objective is ten, and that the lens therefore is correctly described as a one-inch objective. Similarly, if we take any other lens and divide the magnifying power obtained by six, the result again will give the initial power of the lens at a distance of ten inches. With higher powers it will be necessary to use the one-hundredth millimetre scale. The reason why it is advisable to take a long camera-extension is that the distance should be reckoned from the posterior principal focus of the lens, and this is not a point easy to determine. Over a long distance the error becomes so small that it is not of great moment, and the distance may be measured from the front lens of the objective.

Another small error is introduced owing to the fact that the further the image is from the objective, the nearer the lens has to be to the object to get the projected image into correct focus, but this again is not sufficient to disturb the result for ordinary work. It must be carefully noted in making any estimation of magnification that when an ocular is used this estimation only holds good for the particular tube-length with which the determination is made. In determining magnification by the aid of a micrometer it is not advisable to rely upon the distance between any two rulings, but rather to measure several and take the mean of these; there is usually some error in individual rulings and this is reduced to a minimum if the mean is taken. Where ordinary oculars are used the determination must be made with each objective in combination with each ocular.

As examples of magnification to be obtained with varying types and makes of objectives, those given in the accompanying table are typical.

These are cited as examples, but it must not be assumed that all objectives even of the same nominal focal length give the same magnification. If the exact magnification is required it must be determined for each combination of objective and ocular by means of a micrometer.

To ascertain the dimensions of a microscopic object, the photographic method is probably the most accurate. If the magnification is first exactly determined it is only necessary to measure the size of the object as seen in the photograph, and then to divide the measurement so obtained by the magnification. The result gives the actual size. With regular recurring structure, such as in diatoms, it is best to measure the distance covered by a definite number of markings—for example, ten—and to divide the result by this number. Any error that there might otherwise be in taking a measurement between any two lines or spaces is by this means considerably reduced. The method of indicating magnification is in diameters, thus × 100 or × 1000 means one hundred or one thousand times magnification respectively. Another method, generally used on the Continent, is to write $\frac{100}{1}$ or $\frac{1000}{1}$, and $\frac{1}{1}$ thus means that an object is shown in its natural size. Some workers advocate

MAGNIFICATION TABLE. III

Objective.	Ocular.	Camera extension in cm. Measured from ocular cap to focussing screen.							
		40	50	60	70	80	90	100	
26 mm.	Zeiss No. 2 Projection	25	32	39	46	53	60	67	Diameters.
17 mm.	,,	42	53	64	75	86	97	108	
16 mm. Apochromat	,,	52	66	80	94	108	122	136	,,
8 mm. Apochromat	,,	102	130	158	186	214	242	270	,,
$\frac{1}{7}$ in. Zeiss Achromat	,,	260	320	380	440	500	560	620	
$\frac{1}{7}$ in. Leitz Achromat	,,	290	360	430	500	570	640	710	,,
3 mm. Apochromat	,,	280	350	420	490	560	630	770	,,
$\frac{1}{10}$ in. Leitz Achromat	,,	330	410	490	570	650	730	810	,,
2 mm. Apochromat	,,	470	590	710	830	950	1060	1170	,,
$\frac{1}{12}$ in. Achromat .	,,	530	660	790	920	1050	1180	1310	,,
8 mm. Beck Apochromat	Zeiss No. 4 Projection	170	210	250	290	330	370	410	,,
3 mm. Beck Apochromat	,,	550	680	810	940	1070	1200	1330	,,
2 mm. Beck Apochromat	,,	850	1050	1250	1450	1650	1850	2050	,,

the use of a scale, usually in tenths or hundredths of a millimetre, which is printed in close proximity to the photo-micrograph, but this involves an additional printing process and has no obvious advantage. In particular cases, where the question of relative size is of importance, the inclusion of a well-known object at the same magnification for comparison is perhaps the most informative method, but the magnification in diameters should also be given.

A point of importance, to which consideration is rarely given is the limit of useful magnification. This is not dependent on the magnifying power of the optical system employed but on its numerical aperture. Photo-micrographs are often produced at several thousand diameters, but it is doubtful whether they serve any useful purpose, although there is no doubt that such photographs impress the inexperienced. They do, however, enable some observers to see easily small details of structure that are not so obvious when the enlargement is less. High magnification is nevertheless mere amplification, it only alters the scale on which objects are shown. It has the further disadvantage that definition suffers, there is no method available by which perfect delineation can be obtained on a large scale. On theoretical grounds, when using visible light and observing an image in the microscope, the limit of magnification is reached at about 800 diameters, but in taking photo-micrographs some further enlargement is permissible and generally advisable. The grain in the photographic plate and in any printing process does affect the result and some further separation of fine structural details is helpful. A simple rule is to multiply the N.A. of the objective by 1000 and to regard this as a useful limit for most purposes. It follows therefore that with an apochromatic objective of 1·4 N.A. the highest useful magnification is 1400 diameters; anything beyond this is of doubtful utility. With lower powers the proportionately smaller aperture determines the limit in the same ratio and is a safe rule to follow.

Whilst the photographic method is reliable for the measurement of large objects or elements of structure, it becomes less accurate in estimating the size of small bodies. The term 'large' or 'small' is here relative, it really refers to bodies or structures which are well within or very close to the resolving power of the objective used. The apparent size of a photographic image is however not only dependent on resolving power, it can also be altered substantially, particularly with images of small particles, by over-exposure or over-development. Thus with transmitted light images over-exposure or over-development of the negative can cause apparent reduction in the size of the image of any small element. This is due to encroachment of the light and consequent spreading of the light action over too large an area. Over-printing has the reverse effect and must also be guarded against. The same effect but in the opposite direction can take place with dark-ground images.

The Dark-room: Its Illumination and Appointments.—The proper equipment of the dark-room is a matter of importance, although it will often happen that some provision in this direction has previously been made; it may be a question of utilising what already exists rather than installing new equipment.

The main questions in fitting up a dark-room are its illumination, water-supply, ventilation, and regulation of temperature.

The illumination is the chief point, as it will greatly influence the photographic results, and must largely be determined by the class of work to be carried out. The usual source of light is a ruby lamp, this consisting of an ordinary white light of low intensity—such as an oil lamp, gas, or electric light—which is enclosed in a light-tight box, the latter having in one or more sides of it a piece of ruby glass. As in photo-micrographic work plates are used which are sensitive to a considerable range of the spectrum, an ordinary ruby glass is not always sufficient ; in fact, some ruby glasses that are sold for photographic purposes are not safe with ordinary plates, especially if these be highly sensitive. No plate is entirely insensitive to any portion of the visible spectrum ; it only requires to be exposed to any source of light for a sufficient time for an effect to be produced. It will be advisable to have one thickness of rather deep ruby glass and to supplement this with another piece of a deep orange colour, or preferably an additional piece of ruby with one surface ground. It is better to have some diffusing medium, such as the latter, rather than plain glass, as the light is thereby better distributed over the working area. The amount of light that is permissible depends entirely on the plates to be used, but in any case it is not advisable to reduce the illumination of the room to too great an extent ; not only does this entail some strain on the worker, but it is also much more difficult to observe the progress of the plate during development if such observation is necessary. In any case, whatever plate is used, means should be taken to shield it as much as possible from the dark-room light during all operations. For bromide-printing or lantern-slide making, one thickness of orange glass will be sufficient, as in neither of these processes are the emulsions very sensitive. In working with panchromatic plates, which are sensitive to practically the whole spectrum, recourse must be had to a green safe-light. Nearly all photographic plates are less sensitive to a narrow band in the spectrum in the region of the green, and it has been possible, by making a dark-room light of such a colour that it only transmits in this particular region, to work in comfort with plates that cannot be safely exposed to any red light. Such green colour-screens can be obtained from Messrs. Ilford or Kodak, and these in practice are the best illuminants for the dark-room obtainable commercially. They may be had in practically any size to suit the particular form of lamp in use. They must not be subjected to any considerable amount of heat, so that the lamp should be one in which the illuminant is not too close to the screen. In some forms of lamp it is possible to place a glass cell in position, and to fill this cell with a solution composed of dyes which transmit the light required. The advantage of such a light is that there is less general absorption and the quality of the light can be varied at will. One form is a cylindrical glass vessel in which an electric lamp is suspended, so that it is only necessary to fill the vessel with a coloured solution to obtain an efficient means of illumination. Alternatively, gelatin or collodion films may be dyed by the method described for making coloured filters, but these require care in use since they are subject to change as the result of either overheating or prolonged exposure to light.

To make a dark-room lamp of the tank form suitable for work with colour sensitive plates, a solution such as the following may be used. To 100 c.cs. of water add one gramme of aurantia, and 0·25 gramme of methylene-blue. The exact strength of solution should be determined by means of a hand spectroscope, as the light transmitted will depend on the thickness of the cell as well as on the strength of the solution; the strength should be such that it transmits a band of the spectrum limited between 5600 to 5800 A°.

The photographic worker who is accustomed to red light in the dark-room gets the impression that green light cannot possibly be safe for his plates; but this is entirely owing to the fact that the eye is much more sensitive to green than it is to red, so that a smaller quantity of green light can safely be used.

Dark-room illumination should never be effected by daylight, as this source of light is too variable, and the harmful effect of daylight on either ruby or orange glass, or on the green glass filters, is very marked. In any case, every few months the dark-room light should be tested to ensure that no alteration has taken place in the quality of the illumination. This may be done by putting a plate into an ordinary dark-slide, withdrawing the shutter half-way, and exposing the plate to the dark-room light for a minute or two ; on development, no difference should be noticed between the part of the plate which has been exposed to the light and that shaded by the dark-slide shutter. It is unnecessary to describe in any detail the numerous types of dark-room lamp to be found on the market, as reference can be made to any dealer's catalogue for such information. One lamp may however be referred to, as it is used by the writers and is entirely satisfactory. Any type of glass or safe-light may be used with it. It is known as the Wratten Dark-room Lamp and is sold by Messrs. Kodak, Ltd. (Fig. 87). The main feature is that no direct light from the electric lamp used reaches the safe-light, it is all reflected from the white walls of its containing chamber. The result is that a soft diffused light is obtained. The method of ventilation prevents overheating of the safe-light, a point of considerable importance, and one that is often overlooked in such appliances.

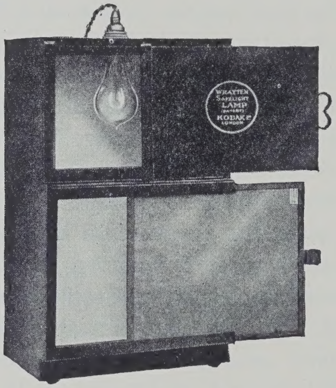


Fig. 87.—Wratten Lamp. (Kodak.)

It is desirable that the dark-room should not be subject to wide variations of temperature. The variation between summer and winter is usually so great that unless some means of regulating the temperature is adopted the time taken to develop plates will vary within considerable limits ; further, the sensitiveness of the plates themselves would appear to be affected to a marked degree, so that the same plate would require somewhat more exposure during winter than during summer. The situation of the dark-room, therefore, should be such that it is not subjected to great changes of temperature, and these conditions can best be obtained if the room is in the basement of a house and has a northern aspect.

A good water-supply is essential, and the tap should give an easy flow, and may be provided with a rose to give a fine spray of water.

It is convenient to have an arrangement for rocking the dish during the process of development, and if a slow developer is being used, it is then quite practicable to place the plate in the developer, cover the dish so that it is protected from light from the dark-room lamp, and start the rocker, leaving it for a few minutes for development to commence. There are many rockers, simple and otherwise, on the market; but a perfectly efficient one may be improvised by anyone with but a small knowledge of carpentry.

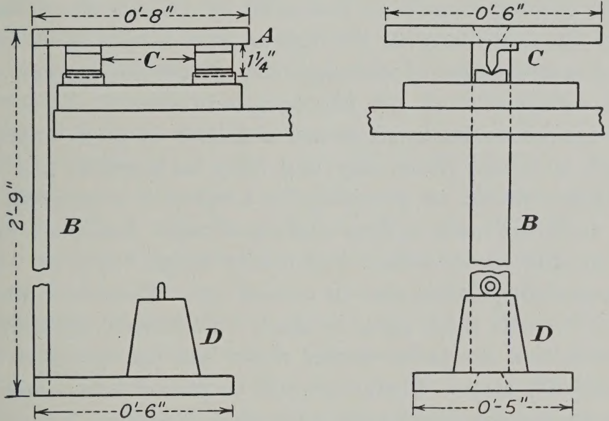


Fig. 88.

Take a piece of any hard wood A about 8 in. by 6 in. by 1 in. and on one of its longer edges screw firmly in a direction at right angles to its surface a strip 3 in. by 1 in., and about 3 ft. long, B (Fig. 88). At the middle-line of the piece of wood

A which supports the dark-room dish, attach two metal angle-pieces C, so that if the arrangement is placed on the dark-room bench it is free to rock on the sharp edges, allowing the long strip of wood B to hang over the edge of the bench. On the lower end of the long strip B fasten a fairly heavy weight D—either a piece of iron or lead—weighing at least seven pounds, and let this be in such a position that it hangs away from the worker, and as nearly as possible under the centre of gravity of the piece of wood supported on the metal edges C. The weight will keep the dish-support horizontal, and a swinging motion imparted to it will continue to rock any dish which is placed on the platform A. The motion being that of a pendulum, the rocking range may be varied by altering the distance between the table carrying the dish and the weight at its lower end. A modification of this appliance is on the market, and may be obtained from any photographic dealer.

The size of a dark-room is not a matter of great moment, although it should be as airy and lofty as possible, and efficient means should be provided for keeping it ventilated. It should be fitted with a deep sink, preferably lead-lined or of porcelain, and at one side a bench may be provided on which development or printing may be carried out. This bench should be covered with thick glass or lead, or alternatively may be painted so that it can be washed down into the sink and thus kept perfectly clean. Shelves should be placed for the various bottles containing developing and other solutions, and these should be quite close at hand. A position should be apportioned for each bottle, so that the worker who is conversant with the arrangements in the room can find any solution he wants in the dark with absolute certainty. It is a good plan also to have the bottles for solutions of varying sizes and shapes, so that after a time the worker becomes intimate with each one without reference to the label. There is thus less liability to use the wrong solution when the conditions are such that the labels on the bottles cannot be clearly seen.

It is all but useless to affix ordinary gum labels to bottles containing photographic solutions, as the constant use quickly causes them to become detached; it is perhaps better with developing solutions to paint on the bottle a number with enamel paint, and to have affixed to the wall of the room a sheet of paper on which is set out the various solutions in their order, with their corresponding numbers.

An ordinary paper label may be made to adhere to the bottle for a much longer time by painting it over with a thin coat of melted paraffin wax. The wax must not be applied until the gum-label has been on the bottle long enough to become quite dry.

A supply of porcelain dishes will be required, and these should be deep and of the best quality. The enamel on cheap dishes soon cracks, and the chemicals that are in use cannot, therefore, be entirely eliminated from the dish as they tend to lodge in these cracks. The dishes should be thoroughly cleansed with dilute sulphuric acid after use, or where they have become silver-stained should be washed with a solution of permanganate of potash. Alternatively the potassium bichromate sulphuric acid solution referred to on page 177 may be used. It is essential that the dishes be kept thoroughly clean, and at the end of the development of each plate the dish should be washed out, never being left with any solution in it. Nearly all photographic solutions tend to oxidise rapidly, and it is often difficult to entirely eliminate them from dishes in which they have been allowed to remain for long periods. A supply consisting of three quarter-plate dishes, and two or three about 8″ × 10″ for printing operations, will be found sufficient. In addition a fixing-bath is advisable. This consists of a porcelain trough which has a rack in it to enable several plates to be placed in it at one time. A fixing solution may be kept in this and used for a number of plates in succession. The plates are put in the trough on edge, and in this position they are fixed rapidly and efficiently. The solution should not, however, be used after it has acquired a distinctly yellow tinge.

Two or three graduated glass measures will be needed: one containing two drachms, for measuring small quantities of solution, and two others of four and eight ounces respectively. These should be thoroughly washed out directly the solution contained in them has been used, as measures are often used for various solutions, an admixture of which would have disastrous results.

As already mentioned, some arrangement must be made to cover the dishes while development is taking place. Nothing is better than an inverted cheap vulcanite or papier-mâché dish, of a larger size than the one actually in use. This will effectually screen the plate from the light of the ruby lamp, or from any stray light which might enter the dark-room.

Draining and drying racks may be provided, in which the plates after thorough washing may be placed to dry. In placing the plates in such racks it should be noted that they do not dry properly if put too close together; hence a rack capable of holding one dozen plates should not have more than three or four plates put in it at once to dry, and these should be so distributed that the greatest air-space possible is allowed between the gelatine surfaces. If dry-plates are stored in the dark-room, care should be taken to ensure that they are kept thoroughly dry, and so far as is possible, in a position in which they are not subjected to any rapid changes of temperature.

It is not advisable to weigh out chemicals or developing agents in the dark-room. Minute particles are very liable to float in the air and may be deposited on plates or papers. Wherever such a particle settles a permanent stain will result. Fixing solution should not be allowed to remain on the bench or splash on the floor. If a plate be removed from the developer or fixing-bath for examination it should be held over the sink so that drops falling from it are washed away. Any drops falling on the floor will dry and become a source of danger.

CHAPTER IX

COLOUR-SCREENS—PHOTOGRAPHIC PLATES—EXPOSURE

Colour-screens.—In photographing microscopic objects a colour-screen is nearly always necessary. It is useful when using achromatic objectives to eliminate that portion of the spectrum for which the objectives are not corrected, or to increase or reduce colour-contrast in the object itself. It will very soon be found that although an object may have sufficient visual contrast, it by no means follows that the same contrast can be obtained on the photographic plate.

The elimination of the harmful portion of the spectrum, so far as the objective is concerned, is a comparatively simple matter, and can be secured by using a yellow screen which cuts out the blue and violet portions. This part is the photographically active portion; but, as in conjunction with such a screen a yellow sensitive plate would be used the exposure would not be increased. To increase the colour-contrast of a preparation is not such a simple matter, and the only effective way is to adapt the screen to the particular dye, or combination of dyes, with which the preparation is stained. Colour-screens may now be purchased which absorb almost any desired part of the spectrum. To purchase such a set of screens is a somewhat costly matter, and many workers may not feel justified in incurring such an expense. Fortunately they may be made up with comparatively little trouble. The matter may be dealt with in two ways. Permanent screens may be made by dyeing either gelatin or collodion films; or, what is much better, liquid screens may be employed, and these may be adapted either in their general or selective absorptive powers to the requirements of each case.

The method of making permanent screens is fairly simple, although perhaps the term 'permanent' is rather misleading, as nearly all aniline dyes are subject to change on exposure to light for long periods. Whether the screen is made of collodion or gelatin will depend on the dye, which may be soluble either in alcohol or water. If soluble in water, the gelatin film may be made in either of two ways. Some of the best hard gelatin may be made up into a 7½ per cent. solution in distilled water. If the screen is being made in very hot weather, it is advisable to make the gelatin solution as strong as 10 per cent. to ensure its setting easily. Take a sufficient quantity of the gelatin, and add water to the strength stated: allow the gelatin to soak for some hours, and then place it over a water-bath until it is completely dissolved. The gelatin may now be filtered through glass-wool or cotton-wool and coated on to small pieces of glass, conveniently about three inches square. This is usually done by pouring on to the centre of the glass a pool of the hot gelatin solution, so that about 2 or 3 c.cs. of solution are allowed to each piece of the size stated; with a piece of glass rod at once cause the gelatin solution to flow evenly over the glass, and place it to set on a perfectly level surface in a position free from dust. As soon as it is set, it may be immersed in a solution of the required dye, until it is stained sufficiently for the purpose in view. The exact strength of the dyeing-solution is immaterial; the stronger it is, the shorter the time necessary to effect the desired staining. On the other hand, too strong a solution of the dye will often stain too rapidly, so that it is difficult to stop at the right moment. In general with aniline dyes, a strength of one in one thousand will be sufficient. On the completion of the dyeing process, the coated glass should be taken out and quickly rinsed in distilled water, then set on edge in a photographic drying-rack to dry.

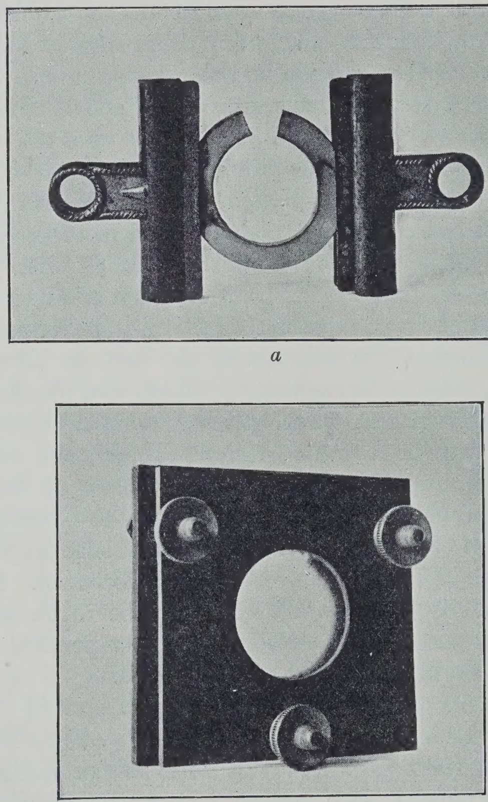
An alternative method is to add the dye-solution directly to the gelatin, and pour this at once on the plate; the disadvantage of this method is that sometimes on drying the screen will be found to have a certain amount of the dry dye, which has separated out, on its surface, and it is consequently less transparent than those made by the first-mentioned process. This may often be remedied by rinsing the screen for a few moments in a dish of distilled water and then allowing it to dry again with the same precautions as before.

If a collodion film is required, the dye must be dissolved in absolute alcohol, and this added to transparent enamel collodion. No ordinary collodion dissolved in alcohol and ether will be sufficiently transparent, and it is necessary to procure the collodion described as ‘enamel collodion.’ A strong or even saturated solution of the dye in absolute alcohol should be used —say, one in a hundred—and a few drops of this added to a small quantity of the collodion and thoroughly mixed. It may then be coated direct on to the glass plates and allowed to dry as described for the gelatin films. To ensure that the collodion film adheres firmly to the glass an edging of a solution of pure india-rubber in benzole may be applied as in the wet collodion process. This can conveniently be obtained in tubes; it is sold for repairing cycle tyres but requires thinning with benzole. None of these dry-film screens transmit quite so much light as a fluid screen, and for this reason the latter is to be recommended.

A cell to contain the solution of the dye, or other liquid colour-screen, may be improvised by taking two pieces of stout glass, preferably patent plate, placing between these a flat india-rubber ring or a piece of india-rubber tube bent into horseshoe form, and clipping the whole together on either side by strong paper clips (Fig. 89, *a*). To prevent bending of the glass, the clips must engage well over the edge so that the pressure is taken up evenly by the rubber between. Such an improvised cell is perfectly efficient, may be taken apart for cleansing purposes, and its cost is but a few pence so that a number may be kept at hand ready for use.

Some excellent cells made entirely of glass may be obtained from R. & J. Beck, Ltd., and from Zeiss (Fig. 90). These are cemented and fused together by what is known as Leybold’s process. The disadvantage with all cemented cells is the difficulty of cleansing them. When a solution which is liable to crystallise out is used—such as a solution of bichromate of potash—the side of such a cell may be forced out as the result of crystallisation. The fluid should therefore never remain in the cell beyond the time that it is in use.

There are many other forms of trough on the market. The one made by Messrs. Watson, and known as Kingsford’s trough (Fig. 91), is very convenient. Its construction is simple, consisting essentially of an outer circle of brass drawn together at the top by means of a screw or screws. This brass circle is lined with india-rubber, and has blocks of india-rubber placed between to keep the circular glass discs or faces in position. The latter are gripped by the india-rubber when tension on the outer metal band is increased by turning the screws, and an absolutely water-tight fitting is the result. It has the advantage that by releasing the screws it can be taken to pieces, either to be cleaned or to replace either of the glasses, should this be necessary at any time.


a

b

Fig. 89.—Improved Glass Cell for Screen.

A simple trough may be made by taking two plates of ebonite

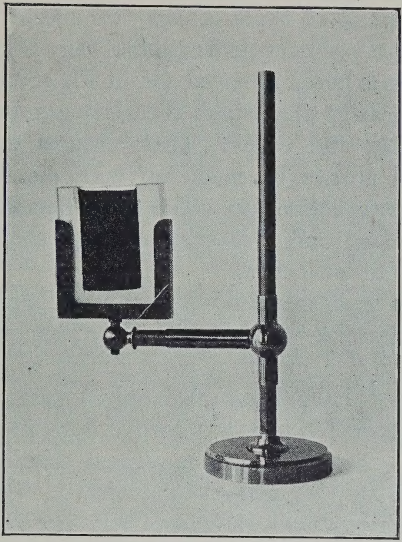


Fig. 90.—Monochromatic Trough.

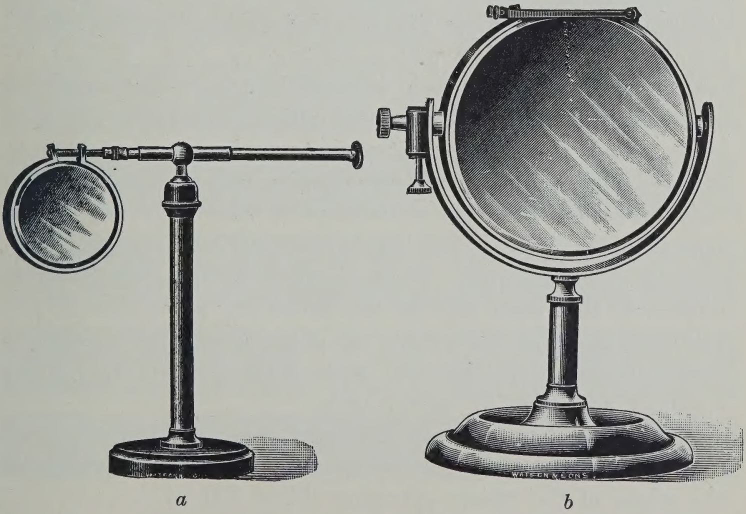


Fig. 91.—Kingsford's Trough for Fluid Screens. (W. Watson & Son.)

—or even hard wood is quite efficient—between which are clamped by means of three screws two pieces of stout glass separated by an ordinary india-rubber ring (Fig. 89 *b*). The rubber ring has a piece removed (as in Fig. 89 *a*) to facilitate filling. By releasing the screws the glass can be removed and replaced quickly and easily. The solutions used as colour-screens should always be made up with distilled water and filtered each time before use. The cells themselves should be scrupulously clean and any solution used should be thrown away if precipitation occurs.

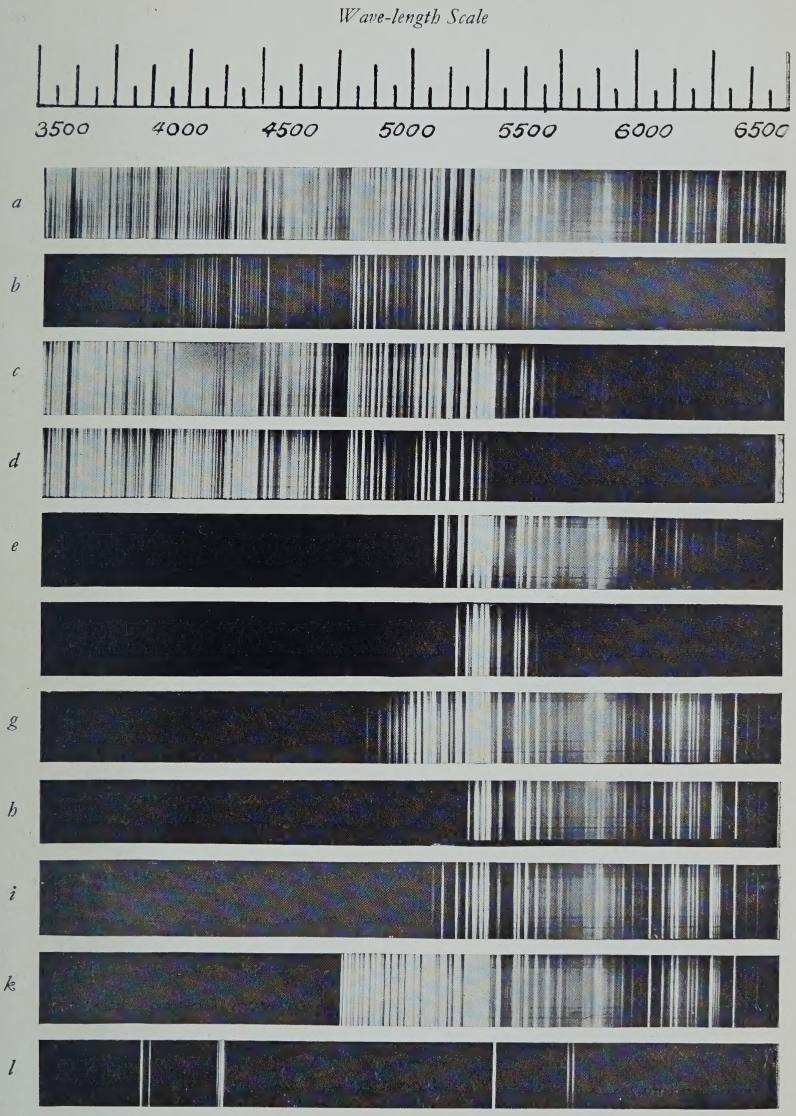
The simplest ready-made filters are pieces of ordinary coloured glass. These may be obtained in a fairly large variety of tints, but their absorption-spectrum often differs widely from what might be expected, having regard only to their visual colour. For ordinary purposes a rather deep yellow, green, blue-green, and blue glass should be chosen. These should if possible be what is known as pot-glass—that is, glass in which the colouring is carried right through and is not merely flashed on to the surface. Such may be obtained from Messrs. Hetley of Soho Square, London. While such colour-screens may answer their purpose and are very convenient, for accurate work they are open to considerable objection.

The illustrations (Plate VII) are photographs of a few typical absorption-spectra taken with a spectroscope with a diffraction-grating mounted on a prism, so that the projected spectrum is practically at right angles to the optical axis of the spectroscope. Such absorption-spectra photographs may be obtained by combining an ordinary long-extension camera with a direct-vision spectroscope. The advantage of using a grating rather than a prism alone is that the energy distribution in the spectrum is more uniform. When a prism is used, dispersion is uneven so that some colours are spread out more than others, this depending on the variety of glass of which the prism is made, but with a diffraction grating the light is distributed regularly according to wave-lengths.

The arc-spectrum of iron has been chosen as the standard, for the reason that it has a large number of lines throughout the whole of the visible spectrum (Plate VII *a*).

Plate VII *b* is the same spectrum taken with a piece of green glass intervening. It will be seen that general absorp-

PLATE VII


Wave-length Scale
3500
4000
4500
5000
5500
6000
6500
a
b
c
d
e
g
h
i
k
l

Spectra of Various Dyes and Colour Screens.

tion of light has taken place, although the exposure was about three times that given to the bare iron-arc spectrum, and in addition the light transmitted is not confined to the green portion of the spectrum only, but extends without any very well-defined boundary in each direction.

Plate VII *c* is taken with the glass known as signal-green. This absorbs nearly the whole of the red end of the spectrum, but it has no particular selective absorption effect on the remainder, and results in considerable loss of light. It is therefore clear that both from the point of view of general loss of light, and from the difficulty of getting glass with a well-defined absorption-spectrum in any particular region, glass screens are not to be recommended. Recourse must then be had to some of the fluid or stained film-screens made with aniline dyes or chemical solutions.

The ideal filter would absorb completely all the region of the spectrum that is not required, and would transmit the remainder with no loss of light. It must be admitted that no known filter conforms entirely to these requirements, and even among the best of them general loss of light is considerable, sometimes amounting to as much as 80 to 85 per cent.

For visual work the best known fluid filter is the Gifford F-line screen (Plate VII *d*). This can be made by dissolving malachite-green in pure glycerin, the strength of the solution being such that it absorbs all the red except one narrow band. Immersed in the solution is a thin piece of optically worked signal-green glass, which must not be thicker than is necessary to absorb the already-mentioned narrow red band; otherwise there is too great general absorption and consequent loss of light. For visual purposes this screen is not to be excelled, but owing to its absorption-spectrum not being sharply defined, it is not generally useful in photo-micrography except when dealing with diatoms.

The figure shows the light transmitted, and it will be seen that it extends well into the violet end of the spectrum.

With achromatic lenses, a series of yellow filters will be found of use; and, providing they are not capable of transmitting any of the ultra-violet part of the spectrum, will ensure a sharp image even if the objective is not photographically well corrected.

A saturated solution of picric acid is one of the best of these.

It has a very well-defined absorption-spectrum, and the whole of the blue-violet and ultra-violet is completely stopped out. It transmits the yellow and green with a minimum of light-absorption.

Of the yellow aniline dyes, the most suitable are filter-yellow K, tartrazine, and aurantia (Plate VII *g*, *h*, and *i*). The strength of solution of any of these, assuming that they are used in solution, must depend on the thickness of the cell; but rather greater efficiency will be secured by using a very thin filter—say, two millimetres thick—and a proportionately stronger solution. Such a filter is conveniently made in the manner described by holding an ordinary rubber elastic band with paper clips between two pieces of glass. A thin rubber band will give approximately the thickness mentioned. When using such a cell the dyes may be kept as saturated solutions in water, and with the above thickness will efficiently absorb the whole of the blue and violet end of the spectrum.

Of green filters there is a very wide selection. The most useful is that known as Zettnow's filter, in which saturated aqueous solutions of copper nitrate and of chromic acid are mixed in suitable proportions. This filter completely cuts out both the blue-violet and the red end of the spectrum. The proportions of the two solutions may be varied so that a greater or less absorption of either end may be obtained at will. The most satisfactory way is to adjust the solution by means of a hand spectroscope until the unwanted part of the spectrum is eliminated.

Another convenient way of making green and yellow-green filters is to keep at hand a saturated solution of acetate of copper and of bichromate of potash; both solutions should be strongly acidulated with glacial acetic acid. They may be combined in any suitable proportion, and will give a strong visual green or yellow-green filter, the predominance of either colour depending on whether there is an excess of the green solution (acetate of copper), or of the orange solution (bichromate of potash). If on combining these two solutions any precipitate is thrown down, it shows that they are not sufficiently acid, and more acetic acid should be added to re-dissolve the precipitate. Plate VII *e* is a photograph of the absorption-spectrum of these solutions combined to give the greatest absorption at the violet end of the spectrum while transmitting as small a proportion of the red as possible.

Of green aniline dyes, malachite-green, methyl-green, naphthol-green, acid-green and iodine-green will be found useful. Naphthol-green is visually somewhat yellow-green or emerald, whereas the other four are blue-green. The strength of solution of these dyes may conveniently be in a proportion of one in one thousand, and the width of the cell in which they are contained should not be less than five millimetres. The absorption-spectra of aniline dyes vary largely with the strength of solution; or, what is much the same in effect, the thickness of cell in which they are contained. A green dye which transmits a specially narrow band, between wave-lengths 4500 and 5000 A° is Victoria green, and a solution of the strength mentioned makes a very efficient filter. Tartrazine and acid-green, in separate solutions, make an efficient and nearly monochromatic-green screen (Plate VII *f*).

There is not such a demand for blue filters as there is for green or yellow, but the most useful chemical solution is ammoniosulphate of copper which efficiently cuts off the red end of the spectrum. Most blue aniline dyes transmit a good deal of red, hence they are not generally used except in conjunction with a screen that will cut off the red portion. A solution of night-blue, one in one thousand, transmits a fairly narrow band between wave-lengths 4000 and 5000 A° and may be found useful under suitable conditions. Messrs. Zeiss have introduced a blue glass which absorbs the whole of the red end of the spectrum; in order to obtain the best result, it should be ordered for and adapted by them to the source of light with which it is to be used.

Methylene-blue transmits a definite band in the red, and must be used in conjunction with a piece of signal-green glass, or some similar absorptive substance which does not allow red to pass. This dye is, however, not suitable for screen making as it bleaches in the presence of light, even if kept in solution under ordinary conditions.

It is obvious that in selecting colour-screens due regard must be paid to the source of light in use. Very weak sources, such as an oil lamp, would only require weak solutions and thin layers to effect the necessary absorption, whereas with an illuminant of high intensity, such as an electric arc, a much stronger solution and a thicker layer must be used.

It is almost impossible to give any absolute directions as to the strength of the solutions in view of these variable factors, and also owing to the fact that aniline dyes, even when described by the same names, are themselves variable. A test should be made with a simple direct-vision spectroscope to ascertain whether the screen in use is absorbing the particular portion that is not required. This is a reasonable precaution to take with any screen to ensure that no change has taken place.

When using a mercury-vapour lamp the conditions are somewhat different from those with any other illuminant. As already stated, the spectrum of mercury vapour is confined principally to three bright lines: one in the yellow—or more correctly, a double line in the yellow—a brilliant green line, and one in the blue (Plate VII *l*). There is also a violet line which is visually not very evident although photographically active. It is therefore only necessary to screen off the lines not required, when the remaining one becomes a truly monochromatic source of light. The dyes that will be required to make a series of filters for this light are:

Filter-yellow K;
Tartrazine;
Acid-green;
Methyl-violet, and
Eosin, soluble in water.

To transmit only the double yellow line, two solutions should be made: filter-yellow K and eosin, each of a strength of one in one thousand, for use in two separate cells five millimetres thick. To transmit the green line only, a solution of acid-green, tartrazine, and filter-yellow K should be used. In this case the three dyes may be made up into one solution, or rather separate solutions made, and equal quantities taken of each. This, as a general rule, is an unsafe procedure with aniline dyes, since they are very liable to interact. The absorption-spectrum of the solutions in many cases alters on mixing, or a precipitate may be thrown down.

To transmit the blue and violet bands only, a solution of methyl-violet, one in five thousand, is sufficient. Owing to the ease and certainty with which monochromatic illumination can be obtained by means of the mercury-vapour lamp, it is for difficult objects, or faintly stained preparations, of the greatest possible service.

Another method of obtaining monochromatic light is a spectroscopic method in which the light is split up, either by a prism or by a diffraction-grating mounted on a prism. A convenient apparatus for doing this is made by Messrs. C. Baker of Holborn. The illuminant is an electric lamp, a very useful form for this purpose being the 4-ampere arc lamp made by Leitz and others. The image of the source of light is projected on to a slit by an achromatic lens ; the beam is then collimated and passed through a prism with a grating mounted on it, the resulting spectrum being projected on to the sub-stage of the microscope by means of another achromatic lens.

By moving the microscope from side to side, any portion of the spectrum may be utilised as required. Provided that there is sufficient dispersion to ensure that the whole of the field is filled with light of one colour only this method is good enough. But it must be admitted that the loss of light is great, and in taking photographs the exposure is unduly lengthened. Further, although the light may be visually monochromatic, it is not even approximately of one wave-length, as in the case of the mercury-vapour lamp.

The use of deep-blue or violet filters is of importance not so much for securing colour contrast, as for increasing resolution in the microscope. Abbe's law determining resolution with a given objective is to multiply the N.A. by twice the number of waves to the inch of the source of light. It therefore follows that approximately twice the resolution may be obtained if light of short wave-length—such as violet—be used instead of the red at the opposite end of the spectrum.

This method is only applicable practically when attempting the resolution of difficult diatoms, or objects with similar recurring structure. In practice, the increased resolution rarely works out at such greater efficiency as theory would indicate, and in general the use of a monochromatic green, or blue-green, light will secure nearly all that is attainable.

The main object of colour-screens in photographic work is to increase contrast. For example, if we have a faintly stained methylene-blue preparation, and we attempt to photograph it on an ordinary plate without a screen, although the visual contrast may be considerable, the resulting photograph, owing to the actinic power of the blue light transmitted, will be disappointing. To obtain a photograph of such a preparation therefore, the use of an orange-colour or deep-yellow screen is indicated. This will effectually block out the greater portion of the blue transmitted, and the image formed will therefore be almost black in appearance, and the resulting photograph will give the maximum obtainable contrast.

To obtain the maximum contrast effect with an object of any particular colour, the simplest way is to ascertain by means of a direct-vision spectroscope what its absorption-spectrum actually is, and to use a screen which as far as possible transmits light only in the particular part of the spectrum that the object absorbs. The example mentioned of a methylene-blue stained specimen with which an orange screen is used, fully carries out these conditions, except that methylene-blue transmits a narrow band in the red; but unless a plate sensitive to the whole spectrum is being employed this is not a matter of moment.

An example in the opposite direction might be the case of a section or specimen stained with eosin, which would visually be of a pale red or pinkish colour. If this preparation were actually only transmitting red, a blue screen would be of use; but on observation it will be found that it also transmits a great deal of blue, so that two colours are transmitted at opposite ends of the spectrum. To obtain the best results in this case, a monochromatic green screen should be used.

To obtain the utmost contrast a general rule may be formulated for either double-stained specimens, or those stained with dyes which transmit light from widely separated portions of the spectrum. In such cases it is a safe rule to use a screen which transmits light that is intermediate in its position in the spectrum between the two colours it is desired to photograph. Since the spectrum colours occur in the order violet, blue, green, orange, yellow, red, it follows that any two of them may be photographed to secure the greatest contrast in each case by using a colour intermediate between them. Should the two colours transmitted lie together in the spectrum, the screen used should be as far on either side as possible.

It may be that the object is not to increase contrast, but to reduce it; although the occasions on which this happens are not so numerous. In such a case, instead of selecting a screen which is intermediate in position in the spectrum between the two colours, one should be chosen which transmits the colour in the object which is the most active photographically. If therefore it were desired to photograph a specimen transmitting red and violet, and to reduce the contrast between them as much as possible, a blue or violet filter might be used; the resulting photograph would then be a reproduction with a minimum of contrast. The latter condition sometimes arises in preparations that are deeply stained, or which do not transmit much light.

A very efficient series of monochromatic filters can be obtained either from Ilford or Kodak. These filters are designed to pass special regions of the spectrum which are as narrow as possible, and the transmission in other regions is reduced to a minimum. The absorption in the region of maximum transmission is designed to be as small as possible, but is still considerable. These filters do all that is claimed for them, but in securing such high efficiency a considerable general loss of light is unavoidable. It is possible to make liquid filters which have somewhat less general absorption, although they may not so efficiently cut out the light that is not required. The series, as sold by Messrs. Kodak, consists of seven filters, and with each one particulars are given of the wave-length transmitted.[1] A simple and convenient holder for colour-screens is seen in Fig. 92. It is intended to take either circular or rectangular screens of almost any size and thickness. It can be supported on any convenient foot and is shown on a saddle for use on an optical bench and on a simple round base that can be placed on any bench or other support. The screen carrying portion is made in **V** form in sections, but one side of the **V** is vertical, the other side projecting outwards at any convenient angle and of any desired width to accommodate screens of varied thickness. This design of holder ensures that the screen is always vertical; it takes up this position when placed in the holder without any adjustment by the user. The screen carrier can be made up from strip metal by anyone with slight mechanical ability.

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[1] An Atlas of absorption-spectra, by Dr. C. E. Kenneth Mees, is published by Messrs. Kodak, Ltd. In it the absorption-spectra of nearly all dyes used in staining microscopic preparations are given, together with photographs of the spectra transmitted by a large series of screens that they now make. A similar booklet entitled *Panchromatism* is published by Ilford, Ltd. It also contains a full description of the filters issued by Messrs. Ilford, together with photographs of absorption and transmission spectra.

In selecting a colour-screen for any purpose due regard must be paid to the photographic plate used. This aspect of the subject will be dealt with in succeeding sections of this book, but it cannot be dissociated from the nature of the microscopical preparation and from the colour-screen used.

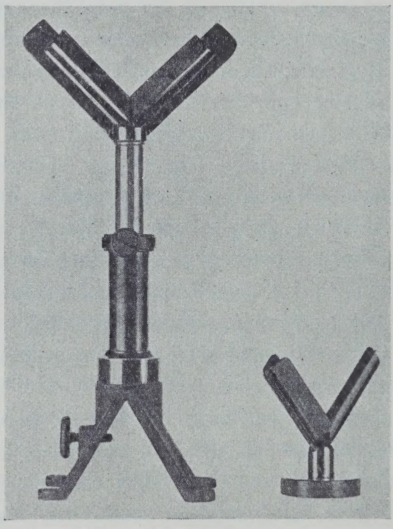


Fig. 92.—Holder for Colour-screens.

Plate VIII shows the effect of varying the colour-screen. Figs. 1 and 2 were taken with fluid screens made up of chromic acid and copper nitrate as already described. The screen used with Fig. 1 contained an excess of copper nitrate, thus the green spiral vessels are subdued but the tissue cells are well shown. Fig. 2 contained an excess of chromic acid with the result that contrast is accentuated between the green and red structures, but the cells are not shown. Fig. 3 was photographed without a colour-screen on an ortho-process plate. The bacilli are almost lost in the general tissue structure although visually

PLATE VIII

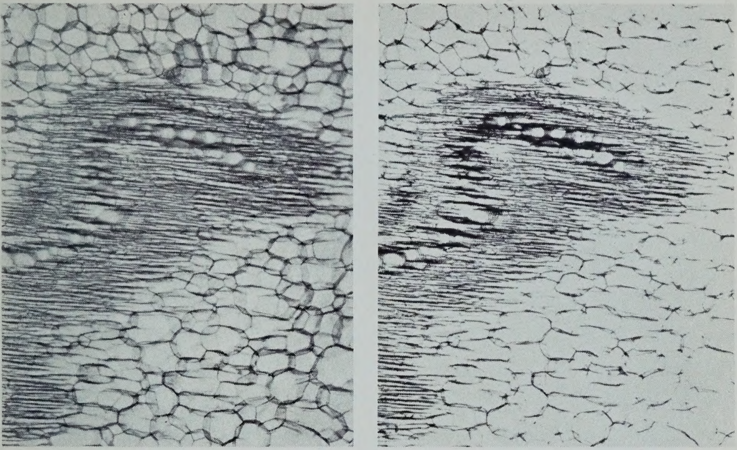


FIG. 1 FIG. 2

Section of the Tree Fern × 45. Stained Carmine and Methyl green. Tissue, red; spiral vessels, green.

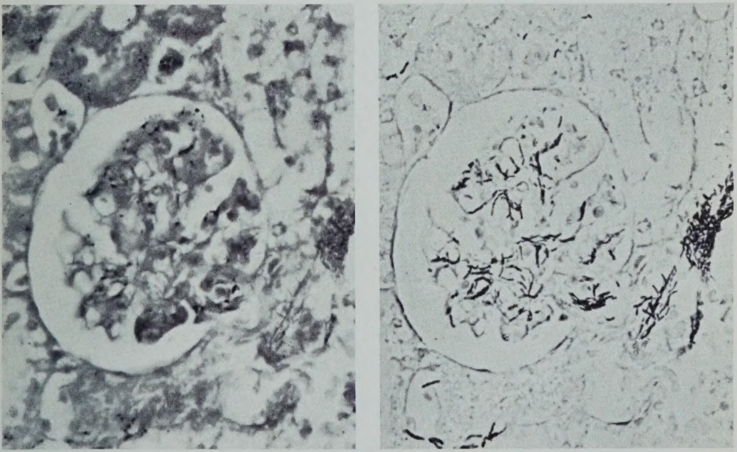


FIG. 3 FIG. 4

B. anthracis in kidney × 370. Stained Gram and Eosin. Bacilli, violet; tissue-substance, pale red.

Effect of Colour Screens

they are very conspicuous. Fig. 4 was photographed with a Wratten F screen (Red) on a panchromatic plate. Here the bacilli stand out and the remainder of the structure is almost obliterated. All these are extreme examples ; some intermediate screen correction would have been desirable to show each element of structure in its proper relation.

Sensitive Plates or Films.—Wet collodion was formerly the only available process, and it must be admitted that in the hands of experts it gave results with some objects that have never been excelled. But the inconvenience of having to coat a plate before each exposure gives an advantage to the gelatin dry plate that to the ordinary worker is irresistible. The essential property of colour-sensitiveness was lacking and this the modern dry-plate fully provides.

In photo-micrographic work a plate should give a bright image with as fine a grain as possible ; that is, however fine the detail in the plate, the grain of the silver particles in the emulsion should not be sufficiently large to become evident. In this respect collodion has some advantage, as the grain of a collodion plate is finer ; in fact the silver deposit partakes more of the nature of a stain than of a definite deposit in the film.

For negative-making, gelatin plates will almost certainly be chosen, and the description of collodion processes will therefore be left until the making of lantern-slides is considered, since it is particularly for the latter that collodion is suited and is employed commercially.

For photo-micrography a plate of medium or slow speed will be found the most satisfactory, since if properly developed, the grain of the image will be as fine as it is possible to get. The method of estimating sensitivity devised by Hurter and Driffield many years ago is perhaps not perfect, but it has not yet been superseded. ‘ The H and D number,’ the term commonly used in referring to this method, is a measure of plate speed and is generally adopted in this country as a guide in estimating exposure. For further information on its principles the reader is referred to any standard work on photography.

Gelatin plates are sometimes described as ‘ ordinary,’ ‘ special rapid,’ or ‘ extra rapid.’ Such plates are usually most sensitive to the blue and violet end of the spectrum—that is, the visually brightest part of the spectrum does not produce the greatest photographic action. Therefore, in photographing preparations in which yellow, green, or red predominate, or where a yellow or green colour-screen is used, an ordinary plate would require abnormally long exposure, and even then the gradation of tone obtained would not faithfully represent the object. To overcome this inequality of sensitiveness, plates are bathed in a suitable aniline dye, or the dye is incorporated with the emulsion in the process of the manufacture of the plate. The result is that plates may be obtained highly sensitive to the yellow and green region, in which case they are generally referred to as ‘ orthochromatic.’ They may be obtained still further sensitised for the whole of the visible spectrum, and are then described as ‘ panchromatic,’ but are still not equally sensitive to all colours.

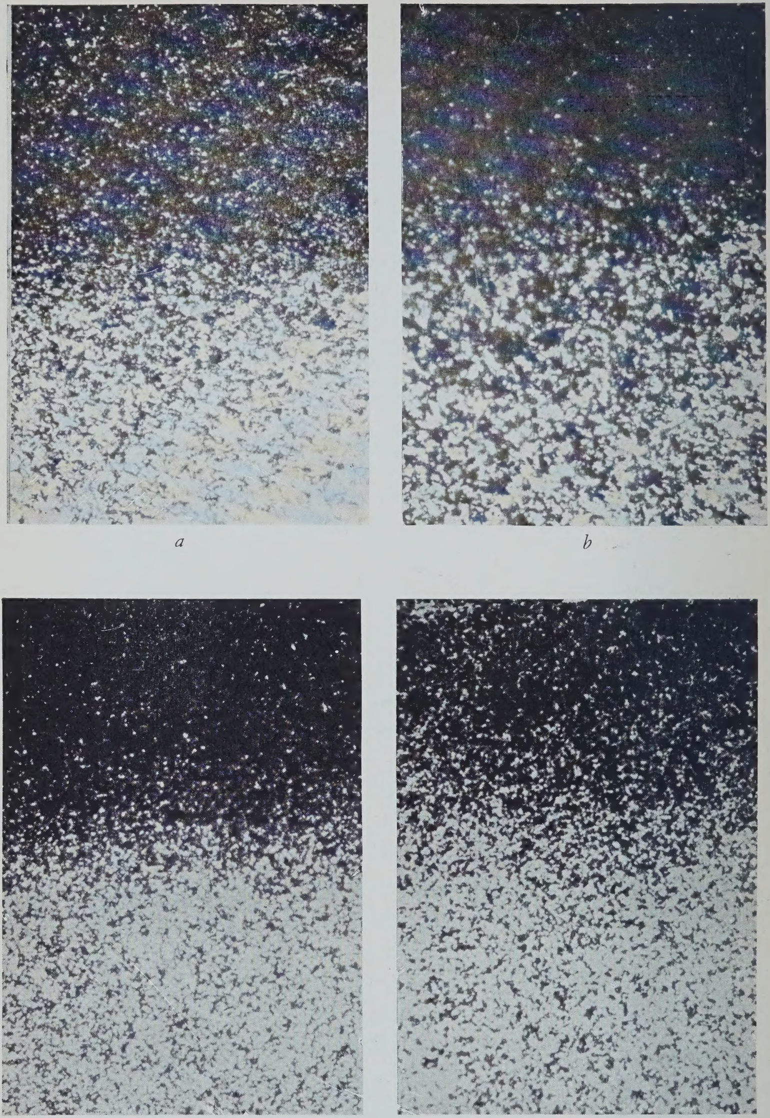
Of suitable plates the following may be taken as typical :

Ilford Panchromatic Process, H & D, 100.
Imperial Fine Grain Ordinary, H & D, 50.
Ilford Orthochromatic Process, H & D, 30.

The above are merely examples ; there are doubtless others which will fulfil the purpose equally well. The plate that the writers have found as good as any is the Ilford orthochromatic process plate ; it is slow, its orthochromatic properties are all that is required, and on development the grain is exceedingly fine.

Whichever plate is chosen, it is important that it should be backed—that is, on the side of the glass other than that on which the emulsion is coated, it should have a layer of some light-absorbing material to ensure that there is no reflection from the back surface of the glass. On any unbacked plates, where there are violent contrasts of light and shade, the effect known as ‘ halation ’ is liable to occur. This consists essentially of a spreading action of the light beyond the margin of the properly defined image, and results therefore in a certain indefiniteness at what would otherwise be an edge of perfectly sharp definition. It is due to the light which passes through the emulsion being reflected from the back surface of the plate. This light is scattered over the back of the emulsion, resulting in some photographic action where it is not wanted. If the reflection of the light from the back of the plate is prevented, the spread-

PLATE IX



a *b*

c *d*

a, Slow backed Plate. *b*, Fast Plate unbacked.

c, Slow Plate Farmer reduced. *d*, Fast Plate persulphite reduced.

Photographs of Grain of Exposed Photographic Plates. × 240.

ing action is minimised. Plates may be procured commercially ready backed, and no extra work is entailed as the backing dissolves in the developer. In photographing objects with very fine detail, there is considerable loss in definition unless the plate is efficiently backed, and this makes it almost impossible to obtain certain results except on backed plates.

Plate IX shows photo-micrographs of a slow backed plate (*a*) and a fast unbacked plate (*b*). The magnification is 240 diameters, and it shows the difference between the size of the grain in a fast and a slow plate. The object photographed is one of the lines in an Abbe test-plate, which appear visually in the negative to be perfectly defined. The dark and light portions in the illustration show that what is apparently a well-defined line is not actually perfectly sharp; but in the case of the slow plate the result is much better than in the fast one. The difference in the grain is at once obvious; but in the case of the fast plate this was also unbacked, so that the spreading action of the light as well as the larger grain is shown up.

Although it has been recognised that the results to be obtained on very slow plates are superior to those on fast ones, yet for some hitherto unexplained reason it often happens that a plate of medium rapidity gives the best result. In a paper by Dr. Kenneth Mees,[1] on the resolving power of photographic plates, it was shown that for rendering of fine detail, more particularly in the applications of photography to spectroscopy, a plate of medium rapidity might be the most suitable. This result is dependent not entirely on fineness of grain, but also on light-diffraction or reflection within the film and between the particles of silver themselves in the emulsion. It is recommended that those who purpose doing work of a critical nature—especially with objects in which there is fine recurring structure—should refer to, and carefully read, this paper.

It was also shown that diffraction and scattering of the light becomes less as the wave-length of the light used increases. It therefore becomes even more necessary to use a suitable plate when violet or ultra-violet light is used as an illuminant. Emulsion is now coated on flat films as well as on glass, nearly all varieties of sensitive material can be obtained on either support.

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[1] *British Journal of Photography*, December 24, 1909.

The advantages of films are that they are unbreakable and easily stored. Further, the halation trouble is not so evident, as the back of the film is near the sensitive surface and the film is not such a good reflecting surface as glass. Films may now be purchased with an anti-halation backing which is discharged on development. For photo-micrography, however, plates are used more than films. Both the Ilford and Kodak Cos. publish excellent booklets on the characteristics and use of their sensitive materials. The Ilford publication, 'Photography as an aid to Scientific Work,' is typical, it not only provides an account of their products but is helpful in the selection of plates for special purposes. Colour-screens are also described with some suggestions on their selection.

Exposure.—Having set up the microscope and adjusted the optical system, it is necessary to determine the exposure to be given. It must be admitted that this is not an easy matter; the determination with varying objects can be arrived at only as the result of experience. No definite rules can be laid down, but in general, exposure depends on three factors:

1. The actinic value of the source of light;
2. The speed of the plate;
3. Magnification.

Other factors have to be taken into consideration in determining the exposure—particularly the character, colour, and density of the object and the colour-screen used. The latter is, however, usually a known constant factor, so that the necessary allowance can always be made for any given screen.

All so-called methods of determining exposure are based on the assumption that the exposure is correct when a sufficient light-action has taken place in parts of the projected image in which there is no light-obstruction. This assumes an uncoloured object which is delineated entirely by perfectly transparent and perfectly opaque areas. The exposure in such a case would be correct if sufficient light-action had taken place in the transparent parts; but, unfortunately, the number of such objects is extremely limited, and in practice the conditions are rarely complied with.

A very simple appliance for estimating the relative intensity of illumination at the position of the focussing-screen is the

Warnerke or Chapman-Jones sensitometer. Appliances similar in principle are the Goldburg wedge, or the step wedges or density meters sold by the E.S.S. Colour Filter Co., Bloomsbury Street, London. These consist essentially of a glass plate divided up into a number of squares or areas which differ in transparency to light. Each one is numbered and bears a definite relationship in its light-transmitting power to the square preceding it; the numbers run with regularly decreasing transparency. It follows that if the sensitometer is placed against the ground-glass focussing-screen of the camera, the image being set up and illuminated, it will be possible to read the numbers on the squares up to a certain point. A number will be reached beyond which it becomes difficult to decipher further. The last number that can be read without difficulty may be taken to indicate the value of the light under those conditions. To obtain more exact indications from the sensitometer when used for exposure purposes, it will be necessary to make some trial exposures. These exposures will form a useful standard for future reference, but they must only be regarded as indicators for exactly similar circumstances. It is hardly a suitable method for use with high-power illuminants, as the range of densities on the screen is not sufficiently great, so that the densest square of the sensitometer can be read with ease. This may be overcome by interposing one or two thicknesses of neutral tint glass between the sensitometer and focussing-screen, so that the power of the light is lessened by a definite amount; the same glass must be used on every occasion when it is desired to estimate the power of the transmitted light. Having determined the correct exposure for any one number it is then easy to determine it for any other visible number as in all such sensitometers the relation of the density of the squares or areas is indicated on the appliance.

The use of the sensitometer is but an approximation, and its readings must only be acted on after due consideration of all the factors that require to be taken into account.

It is obvious that the working N.A. of the objective and of the sub-stage condenser greatly influence the question of exposure, as on these factors the light-transmitting power of the optical combination depends. It has been suggested that exposure might be determined solely in relation to this actual working N.A. While this method may work well, as indeed do most methods of estimating exposure, with a range of objects not presenting any considerable colour or contrast difficulty, it would be of little assistance when widely varying objects are dealt with. The difficulties of exposure are most in evidence to the beginner; the last mentioned method may be commended as enabling him to obtain a series of records that would form a basis for approximate estimations. The method is practically the same as that for determining the illumination of areas at different distances from a given source of light; a method which depends upon the fact that intensity of illumination varies as the square of the distance. Hence with a N.A. of 1·0, and a magnification of 100, an object would require one-fourth the exposure that it would need with a magnification of 200, and one-sixteenth of that required with a magnification of 400, assuming that all other factors remain the same. It is not a difficult matter, where the working N.A. is known, to formulate a table from a few trial exposures which will sufficiently indicate what is needed with varying magnifications.

The weak point is that it is not always easy to estimate the working N.A. To remedy this and supplement the method, Dr. Duncan J. Reid has suggested that this factor may be determined by measuring the diameter of the Ramsden disc above the ocular. The Ramsden disc is that point above the ocular where the rays of light converge to a point after emerging, and the diameter of this disc varies with the aperture of the objective and the power of the ocular. The greater the magnifying power of the latter, the smaller does this disc become. If this small disc is projected on to a transparent millimetre measure, and by means of an ordinary hand-magnifier its size is read off—say in millimetres and tenths—it is possible to determine its diameter directly. Half this diameter should be multiplied by the magnifying power of the ocular (which is usually known), and the total divided by the focal length in millimetres of the objective; the result gives the working N.A. of the objective in use. As an example, assume that a 4-mm. objective, with an ocular magnifying four diameters, is being employed, and that the diameter of the Ramsden disc of the ocular, obtained by direct measurement, is 1 mm. This, multiplied by the power of the ocular (4), gives 4 mm. as the diameter of the objective beam; half of this, 2 mm., is the semi-diameter of the beam, which, divided by 4, the focal length of the objective, gives 0·50 as the N.A. in use at the time. It is again necessary in this case to make some trial exposures, and to determine with a given illuminant the correct exposure for a colourless object; from one or more trials a complete table may be worked out which would give the exposure under varying magnifications.

This method appears to be the most scientific that has yet been suggested for estimating exposure; but here, again, the same objection applies—that it is left to the discretion of the worker to determine the allowance to be made for colour or density differences in his object. With the same illuminant, however, and with one that can always be worked at the same intensity, it would appear to be of considerable service.

If determination of the actinic value of the projected image is to be made, the most practical method is to employ either a Wynne or Watkins exposure-meter. This is the method employed in ordinary pictorial photography, and under proper conditions may be used with considerable accuracy in photomicrography. It depends on observing the actual effect of the light on paper coated with a sensitive emulsion. The paper is allowed to darken until its tint exactly matches that of a tinted standard supplied with the appliance. For photo-micrography the type to use is that made for estimating the exposure for ordinary photographic enlarging. Using this in conjunction with a source of light of moderate or high power—such as the electric arc—the actinic value of the light may be determined with considerable accuracy. In this case also some trial exposures would need to be made, and from such trial exposures a table may be drawn up for future reference. The actinometer must be exposed to the light in exactly the same position that the image will occupy on the screen, the ground-glass screen being removed so that the light is projected directly on to the exposure-meter. In the watch-form of meter, there are two standard tints, to either of which the sensitive paper may be allowed to darken until it matches. The lighter one is the more convenient to use with artificial light as the time occupied is shorter, being in fact one quarter of that necessary to acquire the darker tint. The exposure necessary will vary directly as the time taken by the paper to darken to the required tint so that one or two correct exposures having been made experimentally, a record may be kept and future exposures varied in accordance with the meter indications. Commonly one source of light will be used, and thus variation due to change of illuminant will rarely occur.

The speed of plate used will greatly influence the time of exposure; but to eliminate any trouble due to this cause it will be wise for one make and variety of plate to be adhered to. The speed-value of the plate is usually indicated on the box by the makers; in fact some makers now determine the speed-value for each batch of plates sent out, so that marked speeds may be taken as indicating very exactly the actual speed value of the plate. Formerly, exposure had to be effected with a considerable degree of accuracy to ensure that a negative with proper gradation should be obtained; but latterly, plates have been made which admit of considerable latitude in this respect. In microscopic work, as in ordinary photography, it may be regarded as a safe rule to expose for the darkest part of the subject—that is, expose for the shadows. But in developing, regard must generally be had for the high lights; extreme density must not be obtained in the high lights and detail in those parts must not be obliterated before that in the shadows is brought out. Exposure should be full in any case; it is better to over expose than to under expose. An under exposed plate is useless, and may be regarded as such without wasting any time in endeavouring to save it; but an over exposed plate, in view of the latitude that now exists, may be suitably developed and result in a good negative.

No indication can be given as to the increase of exposure necessary when using colour-screens. For ordinary photography the yellow screen which is supplied commercially increases exposure by daylight from two to ten times, depending on its depth. Should such a screen be used, the same factor will to some extent apply, but will vary according to the illuminant. Where screens of varying colours and varying absorbing power are used, the only practicable way is to determine by trial exposures what the increase is. A record may then be made and allowance made for such increase when exposing. With some screens the increase is not very great. For instance the Gifford

F-line screen, which visually is not very bright may only increase exposure from four to five times owing to the fact that it transmits a good deal of the actinic portion of the spectrum. On the other hand, with deep screens of a yellow-green variety, an increase of forty to fifty times is by no means unusual.

In general it may be said that with the source of light generally in use and with a given plate, a systematic series of exposures made on objects presenting no colour difficulties, will afford more information—especially if records are kept of the conditions under which the photographs are taken—than any amount of instruction.

On optical grounds exposure varies as the square of the camera-extension, that is doubling camera-length means four times the exposure. Exposure also varies inversely as the square of the diameter of the Ramsden disc, and this applies for any objective, ocular, tube-length and sub-stage condenser. These two factors can therefore be considered together, but they cannot be a guide to exact exposure unless due regard is paid to the other factors involved and to which reference has been made. Recently a much greater interest has been taken in the use of exposure meters in ordinary photography, and there is little doubt that any of these can be used in estimating the time of exposure in photo-micrography. The introduction of photo-electric cells represents the greatest advance, although even these have disadvantages of their own and are expensive. The alternative to these is a meter of the tint-matching type, already referred to, or those known as ‘Extinction meters’ of which the Justaphot is an example, the Lios being perhaps the best of these. As the original Warnerke or Chapman-Jones sensitometer is not easily obtainable, an alternative is the ‘Drem-meter’ Print Meter sold by the Westminster Photographic Exchange. In any case considerable allowances must be made in dealing with microscopic objects, as there are such wide differences between them, and even between different parts of the same object. Further, the result desired may not be the same even in any one object. At the risk of some repetition it is advisable to summarise the points to be regarded to simplify the estimation of exposure:

1. Use one light-source and determine by test at intervals that its constancy is maintained.

2. Vary camera-length by definite amounts so that the variation in light intensity due to differing magnifications may vary regularly.

3. Use the smallest possible number of objectives and oculars and remember that the factor determining exposure is N.A. rather than focal length.

4. Use the sub-stage condenser at the largest aperture that the objective will tolerate. In most cases this will mean that the condenser is working at about three-fourths the N.A. of the objective. Avoid much variation from this even to obtain penetration.

5. Use plates of known speed and do not, unless for some very definite reason, be tempted to try various makes and types.

6. Adopt one method of development and endeavour to keep the conditions constant.

CHAPTER X

PHOTOGRAPHIC PROCESSES

Negative Development.—The method of developing photomicrographic negatives does not differ from that adopted in ordinary photography, and the reader is recommended to refer to one of the many text-books on pure photography for more detailed instructions. Particularly if this is the first attempt at any photographic process, recourse must be had to a simple text-book.

The process in general is as follows. The plate, after exposure, is removed from the dark-slide in the dark-room, and is at once without any preliminary washing put into what is known as the developer. This acts on the plate, and the result of the exposure becomes evident in a shorter or longer time. When development is completed, the salts of silver unacted on and remaining in the film have to be removed; this process is known as fixing the plate. After fixation is complete, the plate has to be washed, until the fixing solution is completely eliminated from the film. The plate is then put on edge to dry, in a position free from dust, after which it is ready for any printing operation.

The number of developers is practically unlimited, and it is best to realise that there is little special virtue in any particular developer, it is rather a question of the manner in which the developer is used. Assuming that exposure has been correct, development may be conducted with comparative ease, and, provided sufficient time is allowed for the developer to act, a satisfactory negative is likely to result. There are three methods of ascertaining when development has proceeded for a sufficient and proper time:

1. By direct observation of the plate during development;
2. By the method known as ‘time and temperature.’
3. By that known as the ‘Factorial’ method.

In the first case the developer is allowed to act until all the detail that is to appear in the resulting photograph can easily be seen on the plate, the method therefore is one in which a certain amount of experience and careful observation are essential. The time-method eliminates the personal factor to a large extent, the plate simply being put in the developer at a certain temperature for a given length of time, and when such time has elapsed the plate is removed from the solution, washed, and at once fixed. In the 'Factorial' method the point to determine is the time that elapses between putting the plate into the developer and the first appearance of the image. This time is multiplied by a factor which depends on the developer being used, and the total time to be allowed is the time that the image takes to appear, multiplied by the development factor. Again, therefore, the personal element does not come in, the method is more or less mechanical. The majority of workers, however, will commence with the first method—in which the image is directly observed—to determine when development has proceeded far enough; and for photo-micrographic work it is sometimes the most suitable method. It may happen that it is not so much a question of bringing out a picture with perfect gradation, as of obtaining one in which there is considerable differentiation between the parts of an object, and for this purpose the observational method is more satisfactory. In all photographic operations the temperature of the room in which the work is carried out, together with that of the solutions, has considerable influence on the result; particularly does it affect the time that the various processes take.

The developers that are most useful for negative-making are pyrogallic acid, hydrokinone and metol.

Every maker places in each box of plates the formulæ with which the particular plates may be developed, and it is obvious that if these directions are followed they will give good results. On the other hand, nearly all plates have a certain amount of latitude, and may be expected to give good results with standard developing solutions.

To reduce the number of solutions as far as possible, the following four may be made up:

Solution No. 1.

Pyrogallic acid	8 grammes.
Sodium metabisulphite	4 grammes.
Water	to make up to 1000 c.cs.

Solution No. 2.

Sodium carbonate	50 grammes.
Sodium sulphite	50 grammes.
Water	to make up to 1000 c.cs.

Solution No. 3.

Metol	4 grammes.
Hydrokinone	8 grammes.
Potassium metabisulphite	4 grammes.
Water	to make up to 1000 c.cs.

Solution No. 4,

Potassium bromide	10 grammes.
Water	to make up to 100 c.cs.

The advantage of these solutions is that they may be employed with any plate. There is the additional advantage that, in suitable combination with one another, they may be used for developing either negatives or lantern-slides, or for prints on bromide paper. The pyrogallic acid and sodium carbonate solution will be found most suitable for negative-making, and to compound a solution for use, equal parts may be taken of No. 1 and No. 2.

Solution No. 4 is a restraining solution only, and where the negative is known to be somewhat over exposed, or where particularly hard contrast is desired, from two to ten drops may be added to each ounce of developer.

For lantern-slides or bromide-printing, equal parts should be taken of solutions Nos. 2 and 3, but this will be referred to later when treating these particular subjects. All these solutions keep well, but they should in any case be kept in well-stoppered or well-corked bottles. It is better not to make up more than is likely to be used in the course of a month or two, and by storing solution No. 1 in small completely filled bottles the chance of deterioration is reduced to a minimum.

When a plate is over exposed, the amount of solution No. 1

may be increased, and that of solution No. 2 decreased somewhat; and the reverse applies in cases of under exposure, No. 2 then being in excess, and No. 1 somewhat reduced. These solutions will be found to act considerably more slowly in cold weather; in fact, when the temperature is low it will be found advisable to have a more energetic developer, as the developing action, apart from its speed, appears to be somewhat retarded. The metol-hydrokinone formula may also be used for negative development, and will be found quite suitable where softness of gradation and full detail is required.

When development is completed, the plate should be washed for a few minutes under the tap, and then immersed in a fixing solution composed of 200 grammes hyposulphite of soda, and water to 1000 c.cs. The exact strength of this solution is immaterial; but the plate should be allowed to remain until all whiteness, as seen from the back of the plate, has completely disappeared. A fresh solution should be used for each batch of plates, and it is better to leave the plate in the solution for several minutes after fixation appears to be complete to ensure that no unaltered silver remains in the film. Incomplete fixation is sure to result in the appearance of markings on the plates at a later stage, or to cause some general deterioration. The plate should not be exposed to white light until fixation is complete. Most plate-makers now recommend an acid fixing solution, particularly when pyrogallic acid is used as the developer. Its purpose is to prevent yellow stain which otherwise may appear. The Ilford formula is:

Sodium hyposulphite . . .	400 grammes.
Potassium metabisulphite . . .	25 grammes.
Water	1000 c.cs.

The hypo. should be dissolved in hot water and the potassium metabisulphite added when it is cool.

After fixation, the plate should be washed thoroughly in running water for about an hour; this time will suffice if washing is carried out efficiently. Thorough washing is as necessary as complete fixation or the same deterioration of the finished negative will occur. The plate may then be placed on edge or in a suitable rack to dry, and this should be done in a place free from dust. The plate should not be moved until it is quite dry, any interruption to the drying process, or a change of temperature, is likely to cause what are called ‘drying marks.’

During the operation of developing, and also, if possible, during fixation, the dish containing the plate should be rocked to ensure even action of the solutions, or one of the mechanical rockers, already described, can be used to effect this purpose.

Fully one ounce of developer should be used to develop a quarter-plate. It is important to see that the plate is well covered by the solution, otherwise air-bubbles may attach themselves to the plate and cause clear transparent spots to appear in the negative.

However safe the dark-room light may be, it is advisable to cover the plate during development and fixation and to shield it as much as possible from light during all operations. For this purpose it is convenient to have a larger dish to invert over the one containing the plate, or a cardboard plate-box may be used to cover the dish.

When pyrogallic acid is used, it is as well to have fresh developer for each plate. Rather rapid oxidation occurs with this developer as considerable staining of the plate occurs if it is used more than once, even if an acid-fixing solution is used. With other developers this is not the case, they may be used for two or three plates in succession, the only effect being that they become gradually slower in action.

It is not always easy to judge whether a plate is over or under exposed. In general, the over exposed plates come up quickly when placed in the developer, and there is a lack of contrast in the resulting negative. With under exposure, although there may be ample density in parts, there will usually be some areas in which there is a total lack of detail. Further than this, it is very difficult to indicate the differences between under and over exposed plates, since the effect will vary so largely with different objects. The over exposed negative is often thin, although it possesses ample detail in every part, and in this case it may be improved by the subsequent operation of intensification. The under exposed plate can rarely be dealt with at all, and unless the negative is one that cannot be repeated, it is better to expose another plate at once.

The appearance of small transparent spots known as pinholes in the negative indicates the presence of dust upon the plate before exposure; it probably arises from the dark-slide or camera itself. Both before exposure and before development the plate should be sharply tapped on the dark-room bench to dislodge any dust particles attached to it. This is a better method than using the dusting-brush or any such appliance, as these often add to rather than remove any adherent dust.

It is better to adopt one standard developer and keep to it, mastering its peculiarities and possibilities thoroughly. The same may be said in reference to plates; select a suitable one, master its characteristics, and there is every probability that good results will be obtained.

In cases of under exposure, where development has been prolonged or has been forced by using an excess of alkali care should be taken to stop the process before general fog appears. The amount of the alkali solution permissible is usually not greatly in excess of the normal amount, and an increase of about 10 per cent. in the quantity is probably the limit. Many workers, who conduct photographic operations at irregular intervals, are troubled by the deterioration of their solutions. This may be remedied in one of two ways. The solutions may be made up in the quantities mentioned, but instead of storing them in large bottles they may be stored in several small ones, which to prevent the access of air, should be tightly corked or sealed down. One may then be opened and as far as possible used up as required. In any case the keeping quality of solutions is considerably increased by boiling the water before making up, so that the least possible amount of air remains in solution. The use of boiled distilled water is to be preferred as with tap-water a precipitate may be thrown down. An alternative method, and one that is now easily available, is to use the tabloid developers made by Messrs. Burroughs Wellcome & Co. Almost any developer can be obtained in tabloid form, and there is the advantage that only a sufficient quantity is made up at a time. The solutions are therefore fresh, do not suffer from deterioration, and work under their best possible conditions. The only point against them is the time taken in dissolving the tabloids when a plate has to be developed, and the slight additional cost of the tabloids as compared with chemicals bought in bulk.

In some cases of under exposure, when it is impossible to secure another negative and forced development is therefore unavoidable, a small quantity (from 10 to 20 drops per ounce of developer) of a 10 per cent. aqueous solution of metol may be added to the pyrogallic-acid developer. This method is preferred by some workers to adding more than the normal quantity of alkali solution; there is less liability of the plates being fogged when development is greatly prolonged. Time development, or what is also known as 'Thermo' development, is the best method for panchromatic plates, as it is unnecessary to expose them to light of any sort. This method, as well as factorial development, is fully described in 'The Watkins Manual of Photography,' which can be obtained from any dealer. This manual is the best published on the subject and the reader is advised to study it carefully. Time development consists essentially in a consideration of the effect of temperature on the rate at which the process proceeds. The time taken at any given temperature is always the same for any one developer, but varies for different developers. Metol, for instance, has a ratio of only 1 to 1·25 for a variation of temperature from 68° F. to 50° F., while the ratio for hydrokinone is 1 to 2·8. The temperature coefficient being known for the developer used, and also the development time for a particular plate at any one temperature, it is possible to draw up a table of times for all temperatures for this plate. Most makers now supply development information with each box of plates sold and some give sufficient information for time development. The method is excellent for those who will take the small trouble involved in mastering it and gives more uniform results than any other. It is particularly suited for photo-micrography as exposures are made by artificial light of known value so that wide variation should be avoided. In making up solutions for this process it must be remembered that the temperature of water taken from a tap is rarely room temperature. The solution must therefore either be warmed or cooled as may be necessary.

The Factorial Method of Development may be described briefly as follows, but fuller details will be found in 'The Watkins Manual of Photography.'

A dark-room clock which can be manipulated in the same way as a stop-watch is a great convenience for this method, although an ordinary watch will serve the purpose.

The clock hands are set at zero. The developer is mixed and the plate put face upwards into a developing dish. The plate is flooded with the developer so that it flows over in one wave. At the moment of pouring on the developer the clock is started. The plate can be watched, taking care not to bring it too near to the dark-room lamp, and at the first sign of darkening of the surface of the plate the time that has elapsed is noted. This ‘ time of appearance ’ is multiplied by the factor for the particular developer in use. The dish containing the plate is now covered with a dish of larger size and development is allowed to proceed until this time has elapsed, the dish being rocked meanwhile. The plate is then rinsed under the tap and put into the fixing solution. The following developer is well suited for this method and will be found of value in photo-micrographic work where strong contrast is desired.

I.	Sodium sulphite . .	100 grammes	Add pyrogallic acid *last.*
	Pyrogallic acid . .	14 grammes	
	Water	500 c.cs.	
	Sulphuric acid . .	6 drops	
II.	Sodium carbonate .	50 grammes.	
	Water	500 c.cs.	

To use take 1 part water, 1 part No. 1, 2 parts No. II. Add 3 drops per ounce of 10 per cent. solution of potassium bromide. Factor for this developer is **4.**

In ordinary photography the tendency now is to use fine-grain emulsions and with them to employ developers that give a fine-grain image. This is the result of the preference now given to miniature cameras which produce a small negative on plate or film suitable for enlargement. This method is also applied to photo-micrography, the Leica camera being the outstanding example. It should be recognised that the use of a fine-grain developer will produce a negative of finer grain with most if not all makes of plates. The essential is that exposure be ample, probably twice as much as that given when an ordinary developer is used. Some fine-grain developers act slowly, the time of development being as much as three or four times the normal. The following developer may be regarded as typical; it has the advantage that its action takes place in normal time. With some the action is rather too slow for general use.

Metol	2 grammes.
Sodium sulphite cryst. . . .	180 grammes.
Hydrokinone	5 grammes.
Borax	2 grammes.
Distilled water. . . .	to 1000 c.cs.

When metol is contained in any developer the constituents should be dissolved in the order in which they are given in the formula.

Intensification.—Some negatives may not be dense enough to yield a good print. It will sometimes be found when dealing with insufficiently stained bacteriological or similar preparations that it is difficult to get a negative with sufficient contrast unless after-intensification of the negative is resorted to.

In some cases where a negative is considerably over exposed and is thin, slight reduction followed by intensification will improve the result.

There are many methods of intensification available; the following will, however, suffice.

The negative is bleached in a saturated solution of bichloride of mercury to which about $\frac{1}{2}$ c.c. of hydrochloric acid is added to each 100 c.cs. of solution. The negative is allowed to remain in this until it is bleached right through to the back, and is then taken out and thoroughly washed. It may be blackened by immersion either in weak ammonia, 10 per cent. solution of sodium sulphite, or any alkaline developer—such as pyro-soda or metol-hydrokinone, already mentioned, and then washed and dried. The blackening by ammonia gives the greatest amount of intensification, but the results are not so stable as with either of the last two mentioned. Intensification with mercury is often very useful, but it results in an increased granularity of the image; in some cases this is so marked that it shows definitely in the print. A more permanent method, and one that gives a finer deposit, is to immerse the negative in the following solution after bleaching with the bichloride of mercury.

Saturated solution of potassium oxalate, 6 parts;
Saturated solution of ferrous sulphate, 1 part.

The ferrous sulphate must be added to the potassium oxalate, otherwise a yellow precipitate is thrown down; but the latter may be re-dissolved by the addition of a small quantity of the potassium oxalate solution. The negative is immersed in this until blackened throughout, and is then well washed and dried. If the increase of density is not sufficient, the operation may be repeated, but each time a thorough washing of the plate must be carried out.

Another convenient intensifier is chromium, which has the advantage that it is permanent. The following two stock solutions should be made up:

A.	Potash bichromate . . .	50 grammes.
	Water	to 1000 c.cs.
B.	Pure hydrochloric acid . .	100 c.cs.
	Water	to 1000 c.cs.

The bleaching baths are as follows:

		C.	D.	E.
A.	Solution .	40 c.cs.	80 c.cs.	80 c.cs.
B.	Solution .	5 c.cs.	20 c.cs.	80 c.cs.
	Water . .	160 c.cs.	100 c.cs.	40 c.cs.

Bleach the negative in either C, D or E solution, wash until the yellow stain is removed and redevelop with amidol. C gives the greatest intensification, D and E successively less. The E formula may be used for bromide prints, and gives a fine black colour. The intensification of the print is not marked but it does improve its colour. Tabloids can be obtained for this process.

Reduction.—In cases where excessive density has been obtained by over-development, the process of reduction may be resorted to. In the class of work under consideration, Howard Farmer's Reducer is perhaps the most useful. It has the particular property of reducing the thin parts of a negative without proportionately reducing the denser parts. The result is that in negatives in which great contrast is required, a better result may be obtained by full development and by subsequently applying the reducer to the fixed and washed plate. The effect is that the dense parts remain practically unaltered, while the thin parts may even be completely dissolved out. The solution is made by taking some fresh fixing-solution and adding to it from ten to twenty drops per ounce of a freshly prepared saturated aqueous solution of ferricyanide of potassium. It is sufficient to estimate the necessary strength by the colour of the resulting solution: it should be of a pale-yellow colour. It is rapid in action, and should therefore be used weak. It must be made up fresh and used immediately.

Where reduction of the dense parts rather than of the thin parts is required, the following solution should be used:

Ammonium persulphate . .	25 to 50 grammes.
Water	to 1000 c.c.

A fresh solution must be made up at the time of use, preferably in distilled water, and a drop or two of sulphuric acid added to each 100 c.cs. The negative must be thoroughly washed before the reducer is applied, and just before the reducing action is complete must be immersed in a 5 per cent. solution of sodium sulphite. The latter is necessary to ensure the stoppage of the action at the right moment, otherwise the process continues after removal from the persulphate solution.

If considerable reduction has been effected, it is advisable to put the plate into a fresh fixing-bath before washing and finally drying.

These two reducers will probably be found sufficient for any contingency that may arise, and it is important to realise the great difference which exists between them in their mode of action. The one acts in a manner that increases contrast, and so is useful where black-and-white results only are aimed at; the other tends to reduce contrast, and may be used in cases where there are wide tone-differences in the negative which render it difficult to print out detail in the densest parts.

Printing Processes.—A satisfactory negative having been obtained, the next process is to obtain from it a positive on paper or glass. To the ordinary photographer there are a large number of processes available; but in photo-micrographic work, where the artistic side need not be considered, only two are in general use:

1. Printing by daylight on gelatino- or collodio-chloride printing-out paper.

2. Printing by artificial light on some type of bromide paper.

Either of these methods gives a print in which the image is on the surface of the paper and not in its depth, so that the maximum amount of detail is brought out.

The first process is that usually referred to as printing on P.O.P. The paper may be bought of the required size, and is placed in a printing-frame with its sensitive surface in contact with the film-side of the negative. The paper should be placed in position in the frame in a dull light and then placed in bright daylight, but not usually in sunlight, to print. The time occupied in printing depends on the state of the light and the time of year, and may vary from half an hour to a day, or even more in winter.

In cases where printing is greatly prolonged, care should be taken that the negative and paper are well protected from damp, as otherwise staining of the negative, which it is impossible to remove, may result. The resulting print can be modified to some extent by the brightness of the light in which it is printed. In dealing with a very dense negative it is sometimes permissible to print in direct sunlight; whereas a very thin negative may be somewhat increased in brilliancy by printing in as weak a light as possible. The printing process should be carried somewhat farther than appears necessary, as considerable reduction of the image occurs in subsequent operations.

The above operation having been completed, the next step is to tone the print, thereby altering its colour, which ultimately may be any shade from brown-red to blue-black. The toning process is best carried out by the solutions recommended by the makers, and the formulæ for these are always enclosed in each packet of printing-paper. Practically, however, all papers of this type may be toned by a solution made up of a 10 per cent. solution of sulphocyanide of ammonium and a 1 per cent. solution of chloride of gold. The print is first well washed in several changes of water until the water ceases to acquire a milky appearance. To 1000 c.cs. of distilled water add 1 c.c. of the sulphocyanide solution, and 1 c.c. of the gold-chloride solution. After washing, the print is placed in this gold-toning bath, and toning is allowed to proceed until the stage at which the desired colour occurs has been passed; that is, the process is allowed to go on farther than appears necessary as some of the acquired colour is lost in fixing. The print is washed in several changes of water, and is then fixed in a solution of hyposulphite of soda—half the strength recommended for the fixation of negatives. The print is again washed for an hour or more in running water, and hung up by a corner to dry. It is important to note that the solution for toning must be used in a perfectly clean dish and measure, so that there is no contamination from developing solutions. The hyposulphite solution must also be quite fresh and not one that has previously been used.

The artificial-light printing method is that usually referred to as bromide or gaslight printing, and from most points of view is to be preferred. Bromide papers are much more sensitive to light and must be dealt with in the dark-room, except that their sensitiveness is much less than plates, and the developing process may be carried out in yellow or orange light. A white paper, and one having a highly glazed surface, should be chosen. Such papers are made in varying degrees of rapidity, and may be described as rapid, slow, and very slow. The rapid papers are those usually sold for enlargement purposes, whereas the very slow ones are known as ‘ gaslight ’ papers. The latter may be handled in any weak artificial light not necessarily in non-actinic light. To obtain the most brilliant results it is, however, better to work in yellow light with any of these papers, as even the gaslight ones are still sensitive enough to become somewhat veiled if exposed to white light for any length of time.

The choice of a rapid or slow paper will depend largely on the negative ; and it is possible by selecting a slow bromide paper to obtain a much brighter and harder print from a weak negative than can be obtained on a more rapid paper. This point is one of considerable importance, and where negatives varying considerably in density are being dealt with, the results may be modified within wide limits by selecting a paper suited to any particular class of negative. As the image on these papers is produced by development, the result may also be controlled by varying the constituents of the developer, or by stopping the action at an earlier or later stage.

The printing process is carried out by placing a piece of the paper, as before, in contact with the negative in a printing-frame, and then exposing it to a suitable artificial source of light. Almost any light-source may be utilised, except that it is generally not advisable to have it of high actinic value. An ordinary gas-flame, an incandescent electric lamp, or an oil lamp, may be used.

To ensure fairly uniform results, some arrangement should be made for making the exposures at definite distances from the light-source. With ordinary illumination the light-action will vary as the square of the distance; so that if the distance of the light-source from the printing-frame is doubled, the exposure will require to be quadrupled to obtain the same result. In practice, it is found that increasing the distance from the source of light tends to decrease its penetrating power, hence a somewhat harder result is obtained by thus increasing the distance even if the proper proportionate exposure is given. No definite rule can be laid down as to exposure for it varies with the source of light and the rapidity of the paper itself. Two or three trial exposures, in conjunction with the instructions given by the makers of the paper, will indicate the time to be given. The results should be recorded so that under the same conditions they may always be repeated.

Development may be effected by almost any developer except pyrogallic acid. Metol-hydrokinone, already mentioned in dealing with negatives, is in general quite satisfactory. Possibly the best developer for bromide work is amidol, as it gives a pure black image, and is free from any tendency to stain. A 5 per cent. solution of sodium sulphite may be made up, and one grain of amidol per ounce of solution added. Amidol does not keep well in solution, so that only enough should be made up to be used at the time. The exact proportion of amidol is not of importance, and it is generally sufficient, after having weighed out the required quantity once or twice, to make an approximate estimate of the amount by taking it up on the point of a knife or on a small spoon.

Neither does sodium sulphite in solution keep well; so that no more of this should be dissolved than may be required for use. No bromide is necessary with amidol developer, since, given the correct exposure, the results are easily controlled. Several prints may be developed successively in the same solution, but it should not be used when the action is obviously slowing down. Discoloration or staining may result as the developer becomes exhausted.

The time taken for development is usually short, but varies somewhat with different makes of paper: as a rule it is complete in from three to four minutes. Although bromide is not essential, it is with some papers permissible to use it to restrain the developing action. It should, however, only be used in small quantity, since otherwise it tends to cause deterioration of the black image—a muddy brown-black colour resulting. A useful adjunct for estimating the time of exposure when printing by artificial light, whether on paper or glass, is a Sanger Shepherd Density Meter. The negative the density of which is to be measured, is placed in the appliance, and the densest part of it is matched to a standard graduated and numbered wedge. A chart is supplied on which all standard makes of paper and lantern plates are set out and to which the wedge reading number can be referred. The required exposure is then directly read off. With any illuminant a trial exposure must be made to ascertain its actinic value. Where a number of negatives are to be printed from, this instrument is of great value and results in considerable economy. The wedge number having been determined, it may be marked on the negative for future reference.

Whichever type of printing-paper is used, providing that it has a glossy surface it is advisable to glaze the print so that the surface is as flat and even as possible. This is best done by allowing the print to dry after its final washing, and then soaking it again for about five minutes in cold water. Take a piece of perfectly scratchless plate-glass, or a ferrotype plate, and thoroughly clean it, avoiding hard rubbing or scratching of the surface. Next rub over the surface of the plate a thin film of a solution made by dissolving white wax in benzol or xylol. Polish the plate, leaving just sufficient of the wax on the surface to prevent adhesion of the print. Lay the wet print face downwards on the plate or glass, cover it with a sheet of dry blotting-paper, and with a roller squeegee apply pressure so that all water is expelled from between the print and the glazing sheet. Place the whole on edge to dry, and if the process has been properly carried out, the print when thoroughly dry will leave the plate of its own accord, or it may be loosened at one corner and then drawn off. The result should be a highly glazed surface, which adds considerably to its brilliancy as a print. A still more highly glazed surface may be obtained by coating the print with a thin layer of collodion. The plate should be cleaned and polished as before and then coated with what is known as enamel collodion. When the collodion has set, the print, which has been soaked in water, is placed in contact with the collodion surface and squeegeed as before; this produces a still more highly glazed surface. The latter process is, however, rarely necessary, as most P.O.P., or bromide papers made with a glazed surface, can be rendered sufficiently glossy without collodion.

As most photo-micrographic prints are glazed they cannot be mounted satisfactorily by any process involving wetting of the paper. Two methods are available: either to use a solution of white shellac in alcohol as a mountant or to adopt what is known as a dry mounting process. The shellac solution for the former should be of the consistency of thin syrup and should be applied in a thin even layer to both mount and print, the two coated surfaces being quickly rubbed into close contact. Dry mounting is the more satisfactory method, but it involves the purchase of a hot press, which must take the largest-size photograph that is likely to be produced. It consists essentially in putting on to either print or mount a piece of tissue which on the application of heat firmly fixes them together. The actual details of the process are supplied by the makers of the press and adhesive tissue; they need not be repeated in detail here. The process can be carried out with an ordinary flat iron heated to about 80° C.; but this is only suitable for small-size prints.

There are now many varieties of printing-papers, giving from the same negative different degrees of contrast and of colour. Many of these can also be obtained with an emulsion coated on thick paper or thin cardboard so that no mounting is necessary. The Ilford Co. publish a descriptive booklet in which these characteristics are clearly set out, and the reader is advised to consult this booklet if any difficulties arise in the selection of a suitable paper for any particular purpose. It is a mistake to endeavour to economise by purchasing cheap paper, in fact there are few processes in photography where it pays to use any but the best materials.

Lantern-slides.—For projection purposes positives on glass are required, and these are generally referred to as lantern-slides. Their production is quite simple, and is essentially the same as the bromide-printing process on paper. As with ordinary negative-making, dry-plates may be used, or the wet-collodion process may be adopted, although this is not used by other than professional workers. If the negative from which the slide is to be made is of the required size then the contact-printing method is suitable when dry-plates are used; but when the negative or the portion required is larger than the size of the lantern-slide, the process known as reduction must be resorted to. Lantern-slides are usually made 3¼ inches square, and, allowing for a margin for binding and masking, it follows that no negative in which the part to be used is more than 3 inches square can be employed for contact-printing purposes. Most makers of plates suitable for negative-making also manufacture plates for lantern-slides. The difference between the two is chiefly that of speed. Lantern plates are always slow and some are even sufficiently insensitive to be handled and developed in any weak artificial light. For photo-micrographic work it is best to use a plate of medium rapidity, as with these by suitable development full detail can be shown while securing sufficient contrast.

The process consists of putting a lantern-plate in a printing-frame in contact with the negative to be printed from, so that they are film to film. This must be done in the dark-room by orange or yellow light. The exposure may be made by any artificial light-source, and its length will depend on the character of the negative, source of light, and the sensitiveness of the plate in use. Makers give in each box of plates a sufficiently complete set of instructions both as to exposure and development.

In photo-micrographic work the slides are usually required to be bright, and as a rule are preferably black in colour. Plates can be obtained to give either black or brown tones, but the black-toned ones are to be preferred. The metol-hydrokinone developer, already described, gives this black tone satisfactorily or an amidol developer may be substituted. A suitable one is as follows :—

Sodium sulphite	50 grammes.
Amidol	5 grammes.
Water	to 1000 c.cs.

This solution should be made up as required.

An alternative, and almost universal developer, is as follows :

Solution 1.

Potassium metabisulphite	2 grammes.
Metol	1 gramme.
Hydrokinone	1 gramme.
Distilled water	600 c.cs.

Solution 2.

Potassium carbonate	60 grammes.
Distilled water	600 c.cs.

For use, take equal parts of solutions Nos. 1 and 2.

Development is carried out in exactly the same manner as in negative-making ; but the process must only be carried far enough to give a bright-looking positive. No part must be so dense that it will not allow light to penetrate from the projection apparatus when it is projected on to the screen. Some experience is necessary before a good lantern-slide can be produced ; but once this experience is acquired, there is no particular difficulty. A good slide should have at least some small part in which on fixing there is absolutely clear glass. Development is usually much more rapid than when making a negative, and the beginner will probably err on the side of over-development.

Fixing is effected in the same way as with the negative, except that fresh solution should be used for each batch of plates, and should be discarded the moment it shows any sign of discoloration. An acid fixing-bath is perhaps the most suitable, as it ensures that no stain results from oxidation of the developing agent. It may be made by adding about 20 grammes of metabisulphite of soda to each 500 c.cs. of fixing-solution.

To obtain a lantern-slide from a plate which is too large to print by contact, recourse must be had to the reduction process. The method consists essentially of re-photographing the negative by means of an ordinary camera. The latter is placed in such a position in relation to the negative that a reduced image is thrown on to the ground-glass screen. The negative is placed in front of the camera, and the latter extended for such a distance that an image of suitable size is seen. The negative may be illuminated by either daylight or artificial light. The image is focussed as in ordinary photography, and the lantern-plate put in the dark-slide and the exposure made. There are many appliances to be obtained commercially for this purpose. They may be had of fixed focus, in which case they are only suitable for reduction from one size of negative, or they may be variable for any size. The important points to bear in mind are that the negative is evenly illuminated, and that the lens used is such that it does not impair the definition of the final result.

Exposure depends on the intensity of the light used ; in the case of daylight, from one to three minutes will usually suffice. Development is carried out exactly as when making a slide by contact. This method is rarely necessary in photo-micrography, so that reference should be made to one of the manuals on pure photography if further instructions are required.

The positive having been fixed, washed, and dried, as in negative work, a mask of black paper with a suitable aperture is placed over it to cut off any unsightly part of the edge of the image. It is then placed in contact with another plain piece of glass of the same size, so that the film and mask are between the two. Lastly, the edges are bound round with adhesive black paper, and the slide is ready for projection. Lantern-slide making presents no particular difficulty over any other photographic process except that absolute cleanliness is even more essential : any small speck of dust or any imperfection in the negative becomes so painfully evident when a slide is projected on the screen, that it is of the utmost importance to ensure that no such blemish shall be present. Probably all dry-plate lantern-slides can be improved by a short immersion in the Howard Farmer reducing-solution already mentioned. This is not to obtain general reduction of density, but to brighten up the image and remove any slight general fog that may be present. It is often useful to apply the solution by means of a tuft of cotton-wool. Thus local reduction may be effected, and it is sometimes of value in enabling a good slide to be produced from an indifferent negative.

A wide selection of lantern plates is now available, but the following are in regular use by the writers:

Imperial Fine Grain Ordinary Plates, H & D, 45.
Ilford Special Black Tone Lantern Plates, H & D, 9.
Ilford Half-tone Lantern Plates, H & D, 10.

The fine-grain plates are relatively fast and are used to give soft results with good reproduction of tone differences. The Ilford Special Lantern Plates give good contrast from average negatives.

The Half-tone Plates are valuable where great contrast is required together with reproduction of fine detail. These plates are best developed with a caustic-soda-hydrokinone developer, particulars of which will be found in each box. All lantern plates should be purchased with an anti-halation backing to ensure that there is no spreading of the light action with consequent loss of definition.

The most perfect method of making lantern-slides is the wet-collodion process, and, as this process lends itself to negative-making, it will briefly be described.

As already stated, the grain of the image in collodion is finer than in a dry-plate; consequently, where fine detail has to be shown, it is still unrivalled. For a busy worker there are considerable objections to it: the plate must be prepared and coated before an exposure can be made, and this entails a considerable amount of additional work as compared with the dry-plate process. Lantern-slides thus made are, however, particularly transparent and brilliant, and the process has the added advantage that where a slide has been made from a thin negative and is lacking in brilliancy it can be intensified to an extent that is impossible with a dry-plate. The necessary operations are briefly as follows. An ordinary glass plate is thoroughly cleansed and coated with prepared iodised collodion, which may be obtained ready for use from most photographic dealers. The cleansing process must be carried out very efficiently, and, if the glass has not previously been used, it must be well washed in hydrochloric acid and water, followed by a thorough rinsing in clean water and dried. Before the plate is coated with the collodion, it must be edged to prevent the film slipping off in the subsequent developing operations. This is done by running round the outer edge of the plate, by means of a camel-hair brush, a narrow edging, about one-sixteenth inch wide, of a 1 per cent. solution of india-rubber in benzol, and this must be allowed to dry before the plate is coated. A number of plates may be so prepared and kept ready for use. The collodion may be purchased ready iodised, and it is better to have some that has been kept for about a month before it is taken into use; in any case it should have been kept long enough to have changed to a sherry colour. The coating of the plate is not difficult when once the knack has been acquired. Take one of the clean and rubber-edged glasses, hold it by the thumb and finger at one corner, and pour on it sufficient collodion to cover about one-third of the plate. Gently incline it to each corner in succession, so that the collodion is spread evenly over the surface. When the last corner has been covered, tilt the plate and pour any surplus collodion back into the bottle. While the plate is draining, it should be rocked backwards and forwards in order to avoid the formation of ridges as the film sets. The coating operation can be performed in daylight, since up to this point it is not sensitive to light; but the next, the sensitising process, must be carried out in yellow or orange light in the dark-room. The plate is now immersed in a silver-bath, made by dissolving thirty grains of nitrate of silver in each ounce of distilled water. The solution should be distinctly acid to litmus, and if it is not so must be made acid by the addition of nitric acid drop by drop. The solution must be filtered before use. After coating the plate with the collodion film it must be allowed to set for from one to three minutes, according to the temperature, but not long enough for it to become dry in any part. The nitrate of silver solution should be placed in a porcelain dipping-bath, such as may be obtained from any dealer. The collodionised plate having set is immersed in the solution, and allowed to rest there for about a minute. The plate should be raised and, if necessary, re-immersed until all appearance of greasiness disappears. The plate may be allowed to remain about a minute after this appearance has been reached, and it will then be ready for exposure. The time it takes to sensitise will vary usually from three to five minutes. The plate is put into a dark-slide, which should have silver-wire corners fitted to it to avoid contact with the film more than is absolutely necessary, and the exposure made.

As in dry-plate work, no definite instructions can be given as to the length of time necessary for the exposure; this will depend upon the light and density of the negative. But, in general, it may be taken that it will be approximately twice as long as for a dry lantern-plate of average speed. A full exposure is advisable, as intensification of an over exposed plate is quite practicable.

Exposure having been effected, the plate is removed from the slide in the dark-room, and development carried out. A suitable developing-solution is as follows:

Ferrous sulphate	1	gramme.
Glacial acetic acid	0·5	c.c.
Alcohol	2	c.cs.
Water	100	c.cs.

The solution should be kept for a few days before use. It tends to improve with age, but should be discarded when turbidity becomes evident.

About 10 c.cs. of this solution is poured on to the centre of the plate, the latter being held between the thumb and finger, and the developer caused to flow from corner to corner. It is important that it should flow over the plate quickly and evenly since the image comes up almost at once. Sufficient solution should be used to ensure this, but not enough to cause any of the developer to flow over the sides. The plate should be slowly rocked from side to side until development is complete, when it should be gently rinsed under the tap and fixed in a solution of hyposulphite of soda of the usual strength. The image will appear much sooner than in a gelatin plate, and will more quickly gain the required density—the whole operation being completed in from one to two minutes. It is important to see that the developer is not allowed to flow off the plate, as the action depends to a considerable extent upon the free nitrate of silver which remains in the film helping to bring up the image. The loss of density in fixing is slight. Exposure and development should be carried through as quickly as possible after the preparation of the plate to avoid partial and uneven drying.

The advantage of the wet plate now becomes evident. It is possible to intensify a thin slide, or one from a flat negative, and to make it almost, if not quite, as good as a slide from a technically good negative. In gelatin plates this is also possible up to a certain point, but the slide is likely to suffer much more from stain and loss of gradation than in wet collodion. Intensification is effected by applying to the slide, after fixation and washing, the following solution :

Pyrogallic acid	1 gramme.
Citric acid	2 grammes.
Distilled water	250 c.cs.

Just before applying this solution to the plate, a few drops of a 5 per cent. solution of silver nitrate in distilled water may be added ; but this should only be done immediately before use, and to such a quantity of the solution as will be used at once. The solution is poured over the plate exactly in the same manner as in development, and the treatment is continued until sufficient density is obtained. Should the solution become turbid before completion it must be washed off and a fresh portion applied. The plate is finally washed under a gentle stream of water and dried.

Should the slide not have acquired a black colour by development, it may be altered by toning in a solution of chloride of gold in distilled water. The strength may be one grain of gold chloride to ten ounces of water. As the film of a collodion slide is much more liable to injury than gelatin, it is advisable to varnish it. Any clear colourless negative varnish may be used.

In the formulæ given in this section, the decimal system has been adhered to as far as possible ; but in one or two cases, as a matter of convenience, this has been departed from. For instance, it is universally the custom to take one ounce of developing solution as the normal quantity for a quarter-plate ; a departure in such a case, therefore, would not appear to be without justification.

A few words may be useful as to the method of projecting lantern-slides. The essentials are a good source of light, preferably one of small area, a projection-lens which is aplanatic, and a screen which has a perfectly dead-white surface, and is opaque. The latter point is in some respects the most important, and it is certainly the one to which least attention is usually given. A small, brightly illuminated picture is to be preferred to a large, badly lighted one, and it therefore follows that, except for demonstrations to large audiences, a picture six feet in diameter will show in any ordinary lecture-theatre much better than one of larger size.

The following are points to remember in connection with all photographic operations :

Scrupulous cleanliness is essential. All dishes or other vessels should be well washed immediately after use, and are therefore clean when wanted.

Complete fixation in fresh solution, followed by thorough washing, should invariably be carried out.

Plates or papers should be dried in as even a temperature as possible and, having been put to dry should not be transferred to another position until drying is complete, or marks are sure to appear.

No dark-room light is perfectly safe, so plates or papers should only be exposed to it when absolutely necessary.

Keep plates and papers in a dry, cool place, and only purchase sufficient for immediate needs.

It is desirable that all solutions for photographic purposes be filtered, either through filter-paper or through a plug of cotton-wool placed in a glass funnel. This removes particles of undissolved chemicals as well as grit or dust. It is recognised that it is not always practicable as the necessary facilities may not be available except under laboratory conditions.

CHAPTER XI

SOME SPECIAL PROCESSES

The Photography of Metal and other Surfaces. To obtain photographs of opaque objects certain special conditions have to be complied with. The main point of difference between these and transparent objects is in the method of illumination. In the section dealing with the various appliances for illuminating objects, some description has already been given of what are known as opaque illuminators, and it is in the correct use of these that the chief difficulty lies. It is also necessary to have in the microscope itself certain modifications of construction to enable the object and the additional optical appliances to be adjusted in relation to each other.

A further difficulty lies in the method of focussing. It is obviously a disadvantage, when an ordinary stand is employed, if the object cannot be focussed without disturbance of the illuminating arrangements. Fig. 93 shows the principle of the method employed when a metal surface is illuminated by means of the vertical illuminator. From this it will be seen that the illuminant A is a fixed point, and that the position of the reflecting-mirror B at the back of the objective should be constant. To enable this to be done it is advisable for the stage of the microscope to have a vertical movement controlled by an accurately made coarse-adjustment, so that the object may be focussed, leaving only the final exact focussing to the fine-adjustment, if such is necessary at all.

A typical instrument of this class is arranged for either transmitted or reflected light (Fig. 94). This is of the usual standard type for photo-micrographic work already figured and described; but it has in addition a coarse-adjustment, which moves the whole of the stage and sub-stage apparatus. It may also have attached to one side of the body-tube a mirror which has universal motion, and is adjustable in the direction of the length of the tube; so that for low-power work illumination may be carried out by obliquely reflected light. The mirror is in the latter case first adjusted in relation to the surface of the object, and a parallel or convergent beam is projected on it and reflected on to the metal surface. The stage should have mechanical motion, and be capable of rotation; in the one shown interchangeable stages are provided, and

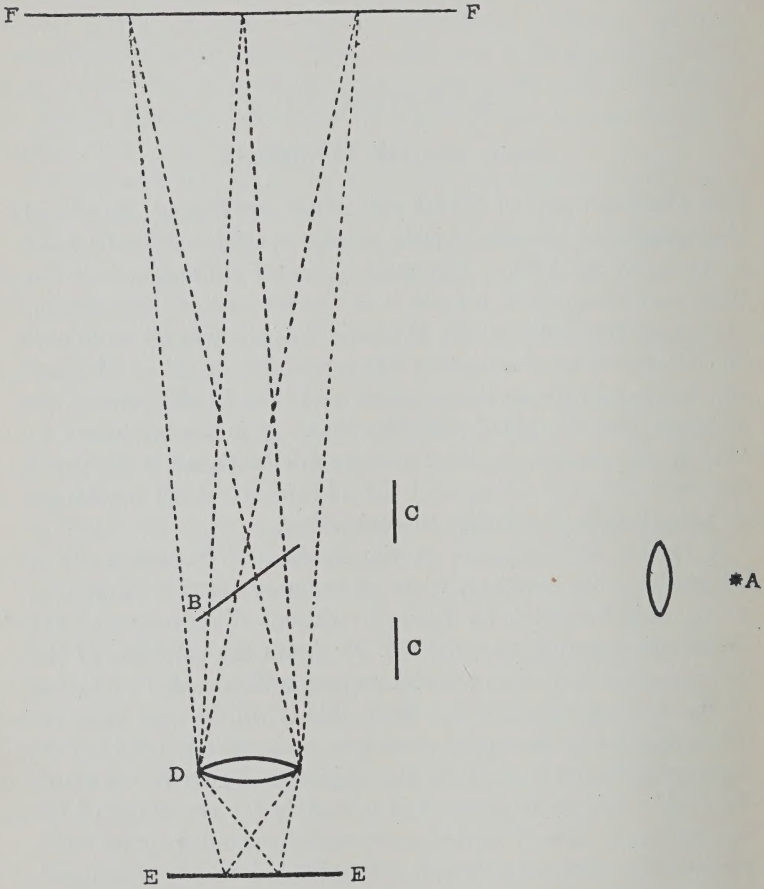


FIG. 93.—Diagram of Path of Rays in Vertical Illuminator.

either can be used on the microscope at will. In metal-work ability to rotate the object is a point of importance, as the direction of incidence of the light in relation to the specimen often has to be varied to bring out some particular structure.

To overcome want of stability in the horizontal position both Messrs. Zeiss and Messrs. Watson & Sons have constructed a stand which is known as Martens' stand (Fig. 95), which differs in design from the orthodox type. The fine-adjustment controls movement of the stage and not of the body-tube, so that in using the vertical illuminator there is no alteration in the incidence of the light. Some modifications can be introduced to enable the instrument to be used for transparent objects; but owing to the fact that it is horizontal it is not so convenient for purposes other than photo-micrography or direct projection. It must be admitted that as a stand for any class of photo-micrographic work—even excluding its suitability for metal-work—it is almost ideal, as the position of maximum stability is attained when it is horizontal; and, owing to the

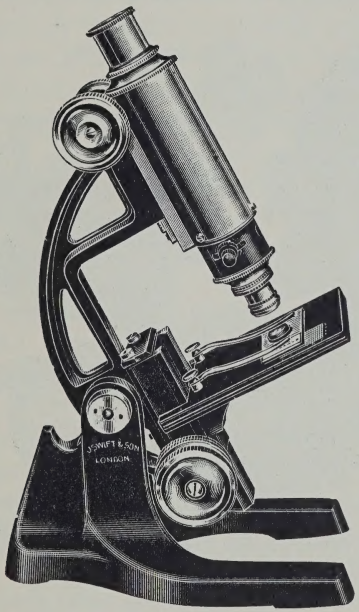


Fig. 94.—Metallurgical Microscope. (James Swift & Son.)

fact that all the adjustable parts are supported in long slides, it is very difficult for any flexure to take place even during a prolonged exposure. For high-power work it is exceptionally stiff and rigid. The method of controlling the fine-adjustment is a direct one; it is connected by a Hooke's joint to a long arm, so that intermediate cords or pulleys for connecting up are unnecessary. Rotation of the stage and mechanical motion are provided; verniers are also supplied, so that any precise position of the stage may be arranged.

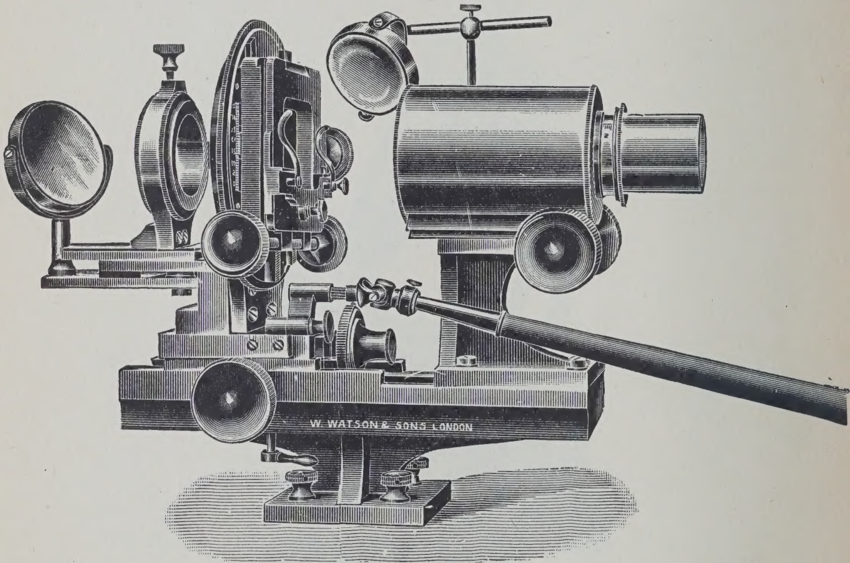


FIG. 95.—Horizontal Photo-micrographic Stand for Opaque Objects. (W. Watson & Sons.)

These instruments differ in important respects from the ordinary microscope, and for this reason they were not dealt with in the chapter devoted to microscope design and construction. As to the objectives for metal-work, they differ in one particular, they must—except in the case of oil-immersion lenses—be corrected to work on objects that are not mounted beneath a cover-glass. To adopt the usual description, they are corrected for uncovered objects. Metals usually have polished or etched surfaces, and when vertical illuminators are used a cover-glass should not be interposed between the objective and the object.

It is therefore necessary for all dry lenses except the lowest powers to be specially corrected. Objectives for this purpose are generally provided with short mounts so that the back lens is as near as possible to the reflector of the vertical illuminator. This is to ensure that the objective is fully filled with light, as otherwise its full aperture would not be utilised. It is often a point of discussion whether a vertical illuminator with a prism or a plane-glass reflector is to be preferred. It should be appreciated that these two appliances are not the same in effect and are therefore not strictly comparable. With the prism form one half of the objective becomes the illuminator and the other half is available for observation; whereas with the plane-glass reflector the whole aperture is used for both, the light is partially reflected, approximately one-half the available quantity being used for each purpose. In most modern vertical illuminators the prism and plane-glass are interchangeable, either can be used at will. This should always be the case, so that both may be tried on any given specimen; it will soon be appreciated that the results are by no means the same in each. The plane-glass reflector should be used for most purposes; the image obtained is nearer to optical perfection as there is no interference with the aperture of the objective. The prism type has its uses as it enhances the differences in contour in an object, but caution should be exercised in interpreting the images obtained. Metal surfaces vary so greatly in the quantity and quality of the light they reflect that no definite rule can be laid down. Objectives of 8 mm. and 16 mm. focal length for metallurgical work are now being supplied as homogeneous immersion lenses, and they should prove of value. It is found that objectives in which the back lens is of bold curvature are more suitable for metallurgical work as the light reflected or scattered by such a surface is less likely to affect adversely the performance of the objective. It has in fact less tendency to cause what is commonly known as glare.

Illumination must be effected on the same general principles as in work by transmitted light; that is, with low-power work a beam must be projected on to the specimen so that the field is evenly illuminated, and in high-power work an image of the illuminant or of the aperture of any collecting lens used must be projected exactly on to the surface of the object. This is in general a matter of no difficulty. The illuminant is placed at a distance of about six to seven inches from the body-tube, and it may then be found that the image of the illuminant is in focus when the surface of the object itself is in focus. Any alteration necessary must be effected by altering the distance of the illuminant from the microscope. Where a collecting-lens is interposed between the source of light and the microscope, it is advisable to use a parallel or only slightly convergent beam. The size of this beam should be cut down by an iris-diaphragm CC (Fig. 93), so that no stray light is allowed to enter the objective. The exact cutting down of the dimensions of the beam is one of the most important points to attend to in metal-work, as with the highly reflecting surfaces of metals, considerable diffusion of light may easily occur. In the later forms of vertical illuminators this is provided for so that the actual size of the beam entering the back combination of the objective may be regulated. The position of the iris-diaphragm is important. If it is near the illuminant or collecting-lens it controls the size of the field. If near the vertical illuminator it determines the working aperture of the objective. This will become clear in succeeding paragraphs.

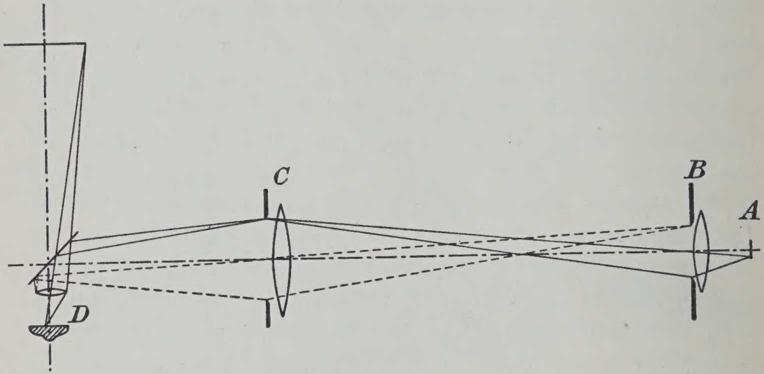


Fig. 96.

The method of illumination used by Mr. H. Wrighton of Woolwich Arsenal has the merit of simplicity and is of high accuracy. It enables a beam of light to be concentrated on the field of view, and is so restricted that no light outside this area falls on the specimen or enters the aperture of the objective. The general arrangement is shown in Fig. 96; A is the source of light, B is a collecting-lens with an iris-diaphragm close to it. An image of the aperture of lens B is thrown by lens C on to the plane of the object D. Both iris-diaphragms at B and C can be contracted so that the illuminated area of the object surface D is strictly limited or the aperture of the objective controlled. To effect this type of illumination the cone of light travelling to and illuminating the object must traverse the same path as that reflected from it—that is, along the optic axis of the microscope. This method is simple in its conception and it results in the projection on the specimen of a disc of light of perfectly controlled dimensions, impinging at such an angle that the aperture of the objective is perfectly filled.

The time of exposure is not so difficult to estimate as when dealing with transparent objects; in fact, so much is this the case that if an object is, for instance, photographed at 400 diameters with an 8-mm. objective and is then photographed at 1000 diameters with a 3-mm. oil-immersion, using a vertical illuminator in each case, the exposure, assuming that all other conditions are the same, will be almost the same length of time in each case. The reason is that the objective itself being the condenser, its working N.A. increases as its own N.A. increases. With the high-power objective the image of the source of light is formed by a wider beam and is of much smaller dimensions when projected on to the object. It follows that the necessary exposure would in each case be almost the same, except that there is some slight difference in the light-absorbing power of the objective itself which must be allowed for. In practice it is found that even with widely varying magnifications, the exposures differ but little when using the same class of objective.

It does not come within the scope of this work to describe the methods of preparing metal surfaces for microscopic observation. In general, however, it may be stated that the object must first be ground or cut so that it has a plane surface. This surface has to be polished so that any scratches or abrasions are obliterated. The polished surface then requires either etching by suitable solvents, or heating and cooling quickly or slowly, so that some differentiation can be seen between the constituents. For further information on this subject the reader is referred to the many books which now deal with the microstructure of metals, particularly the works on 'Alloys,' by Mr.

E. F. Law, 'Metallography,' by Dr. C. H. Desch, and 'Practical Microscopic Metallography,' by R. H. Greaves and H. Wrighton. A second edition of the latter work is now available and brings the subject quite up to date.

The object having been prepared, the first point is to ensure that the polished surface is at right angles to the axis of the microscope. Metal preparations are often irregular in shape, and only one surface—and that the one under observation—is flat. This therefore must be set up exactly in the position stated. The simplest method is to lay the specimen, with its polished side downwards, on an ordinary 3″ × 1″ microscope slip. Around it place a metal ring with parallel ends (such metal rings may be obtained commercially in varying sizes and depths for this purpose from Messrs. R. &. J. Beck), the depth of the ring being greater than the height of the specimen as it lies on the slip. Now place over the specimen some plasticine, putty, or moulders' clay, or some similar easily moulded material. Press down another slip on to the top of this clay so that the object is enclosed between the two slips, these being parallel, and then remove the bottom slip and the metal ring. The object will now be supported on one slip with the polished surface in such a position that it can be placed on the stage of the microscope. This method is simple and inexpensive, and so far as the experience of the writers is concerned, there are few objects that cannot be mounted satisfactorily in this way.

Various appliances are on the market for holding the specimen and adjusting it exactly at right angles to the optic axis of the microscope without the necessity of embedding it in the manner described. Where fractured unpolished surfaces have to be dealt with, or where the incidence of the light has to be varied to suit a particular spot in a specimen, these are of considerable value. In general, however, the method of embedding in some soft material is more useful, as it admits of variation to suit the class of specimen. The only advantage in using some of the devices which are made for holding specimens is that they do not then require any mounting; they can in fact be removed from the device or re-introduced at will. There is the disadvantage, however, that on each occasion they have to be re-levelled, and in practice this is often a much longer operation than to embed again as first described.

To protect a polished surface against tarnishing, the best preservative is a weak solution of celluloid in amyl acetate. When this is gently brushed or poured over the surface, it quickly dries, leaving an extremely thin film of celluloid, which efficiently protects without materially interfering with the corrections of an objective should it be desired to observe the object again without cleaning the surface.

For very low-power work, where illumination is by light impinging in a direction at right angles to the surface of the specimen—a simple appliance, consisting of a piece of optically worked glass held in front of the objective at an angle of 45°, may be used (see Fig. 32). In such a case it is better to use objectives corrected to work with covered objects. The image is formed through the reflector, and where an objective is corrected for use without a cover-glass it may result in some deterioration of the image. Since, however, this method is only applicable for use with very low powers the disturbance is rarely serious. As to the illuminant, any already described may be used, but the electric arc or the Pointolite lamp are best for this class of work. Unless the illuminant is of high power, it will result in lengthened exposures. Incidentally, also, the difficulty of focussing when the image is projected on the ground-glass screen of the camera, and of ensuring even and efficient illumination, is increased unless the illuminant is one of high power. Colour-screens may be used as in work with transmitted light, except that some observation will be necessary to determine the exact effect of the colour-screen in use. When dealing with reflecting surfaces the effect on the image of a given screen is often different from that obtained by transmitted light, and due regard must be paid to this point when setting up the object.

Recently several appliances of a new type have been introduced for illuminating opaque objects by incident light, and the two now described, made by R. & J. Beck, Ltd., are typical. They illuminate the object from all sides so that the resulting image picture is different and in fact complementary to that obtained by a vertical illuminator.

The Beck Aplanatic Ring Illuminator (Fig. 97) is a reflector of the Lieberkuhn type, but, instead of being a silver spherical mirror, is a glass ring mounted around the object-glass with its back surface silvered. The light entering this reflector is refracted at the front surface, reflected at the back surface, and refracted again at the front surface upon emergence. By means of the two refractions and one reflection the ring can be accurately corrected for spherical aberration and the whole of the light received from the mirror is concentrated upon the object; moreover, the size of the reflector compared with the focal length is so large that the whole of this light is delivered at an angle great enough to enable it to enter the small space between the object glass and the object. Two forms of this illuminator are made, one of which delivers the light between the angles of 0·75 and 0·9 N.A. and the other between the angles of 0·86 and 0·95 N.A. The former fully illuminates objects used with lenses with numerical apertures up to 0·7 N.A., the latter those with numerical apertures up to 0·85 N.A. The lenses require to be specially mounted in order that the metal mount shall not obstruct the beam of light.

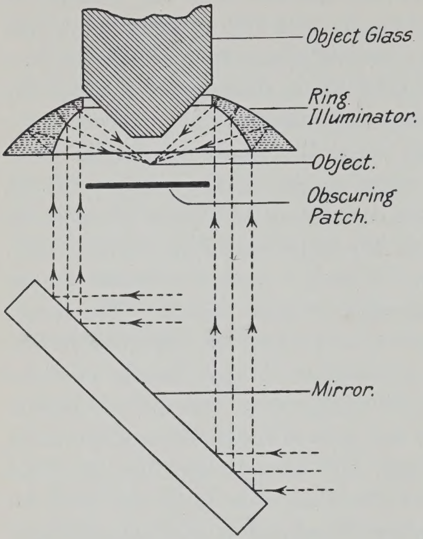


Fig. 97.—The Beck Aplanatic Ring Illuminator.

A development of this method of illumination is its application to oil-immersion objectives with apertures up to 1·3 N.A. For this purpose a special immersion objective is made in which the whole front mounting is made of glass instead of metal (Fig. 98). The glass mounting has an outer surface which is spherical in shape

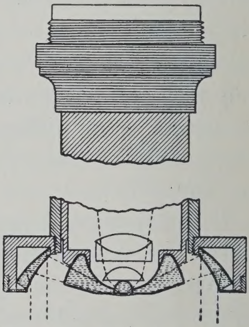


Fig. 98.—Beck Immersion Metallographic Objective.

and concentric with the focus of the objective so that the light from the aplanatic ring illuminator passes through without refraction. The glass front mount has a small aperture in its centre and into this aperture the front lens of the objective is cemented, so that the portion of the front lens usually employed for mounting it into a metal cell is used for transmitting light to the object. The front surface of the front lens is level with the front surface of the glass mount and when in use the whole of this surface is in immersion contact with the object. This development extends this form of illumination to high-power objectives of large aperture. The main objection to ring-illuminators of the types described is that they can only be used with small specimens; there must be room for the light to pass equally all round the object.

Improvements in Vertical Illuminators have also been introduced by R. & J. Beck, Ltd., and W. Watson & Sons, full particulars of which can be obtained from the makers. Both these firms, as well as E. Leitz, Zeiss, Busch and Reichert have also produced photo-micrographic appliances of improved design which embody ingenious methods. These are, however, of an elaborate and expensive character and are intended for those who require facilities for the rapid production of photo-micrographs in numbers.

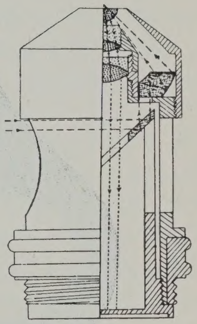


Fig. 99.—Chapman & Alldridge Objective.

A new type of objective for metallography was introduced by Chapman & Alldridge, Ltd., London. These objectives combine a dark-ground illuminator, such as is used in the sub-stage of a microscope, with an objective which is inserted in the centre of it (Fig. 99). A reflector above the objective, as in an ordinary type vertical illuminator, throws the incident light into the illuminator but not into the objective. This is accomplished by having an aperture in the reflector rather larger than the back lens of the objective. A tubular stop projects through the aperture in the reflector to prevent reflection into the eye-piece of the microscope. These objectives give a dark-ground illumination effect, the image being free from stray reflections. It will be evident that the resulting image is a reversal of that obtained by a vertical illuminator.

The principle of this objective has been applied by E. Leitz in the production of a combined objective and illuminator which is called the 'Ultropak-Illuminator.'

This appliance can be used not only for the observation of metallic surfaces by incident illumination, but of any opaque object where the investigation of surface structure is needed. A complete range of objectives is provided, from the lowest dry powers to water and oil-immersion lenses of the highest available apertures. The general arrangement of the apparatus as fitted to a microscope is seen in Fig. 100. The source of light for visual work is an eight-volt lamp which is embodied

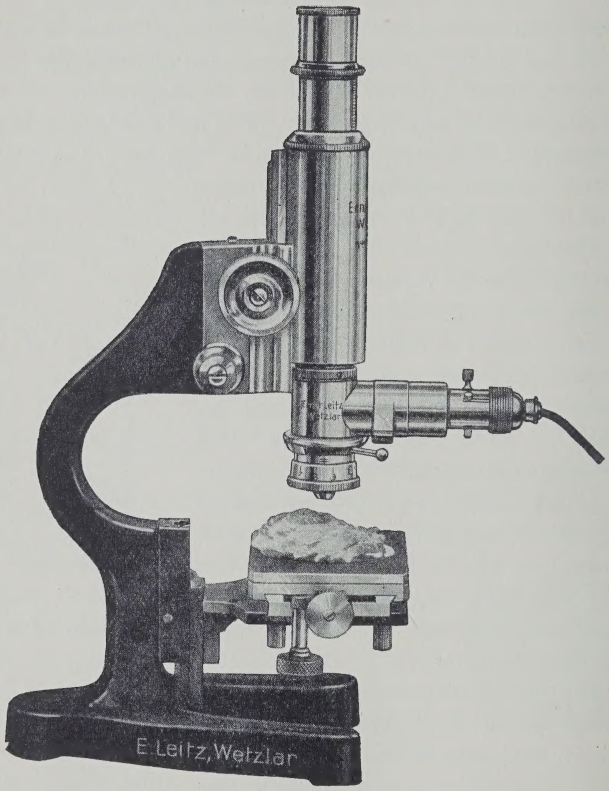


Fig. 100.

in a centring mount and forms a part of the appliance. If other sources of light are to be used, an attachment is provided which replaces the eight-volt lamp fitting. Thus an arc-lamp can be used and instantaneous exposures made should the nature of the object demand it. The improvements introduced by Leitz have resulted in a convenient and useful apparatus which has numerous applications in many branches of microscopy. In metallurgical work it can be used as an alternative to a vertical illuminator, or can be interchangeable with it. Reference should be made to the descriptive booklet issued by Messrs. E. Leitz for full particulars both as to construction and use.

Recently some objectives have been made by Messrs. Carl Zeiss which apparently fulfil the same purpose, and Messrs. R. & J. Beck, Ltd., of London, are now making a full series of objectives, combined with illuminators, on the principle of those originally designed by Chapman & Alldridge.

There is an apparatus for micro-metallurgy, devised by Mr. H. Wrighton, which provides convenient and accurate adjustments. This apparatus has been made by Messrs. R. & J. Beck to a specification prepared in the Research Department, Woolwich Arsenal. It is intended to be used chiefly for metallurgical work, but full provision is made for the micrography of transparent objects.

The requirement chiefly responsible for a new design is the provision of vertical illumination for metal specimens when photographed at low magnifications using short-focus lenses of the photographic anastigmat type. The older types of ‘ metallograph ’ had no special equipment of this kind, and low-power micrographs were taken either under oblique illumination, or with vertical illumination by incident light passed through the objective as in medium and high-power work—a method which is unsatisfactory with powers below one inch.

The conditions of vertical illumination with medium and high powers, Fig. 101, and with low powers, Fig 102, are shown. At medium and high magnifications, the field of view is small in relation to the front of the objective AB (Fig. 101), and may be represented by a point C. Light incident along a path AC is reflected along CB and vice versa, every point of the front lens receiving light from C. In this way the full aperture is utilised for the imaging of every point on the specimen which comes in the field of view; illumination is uniform over the field, and true imaging of fine detail is thereby attainable.

With lower powers, the field of view is large, consequently even lighting of the field and proper utilisation of the aperture cannot be obtained by passing the incident light through the objective. The conditions are shown in Fig. 102; if the extremities of a field BC are to reflect light into the whole front of the objective, a cone of light must be provided wide enough to allow rays corresponding to BD and CE to be reflected. Such a cone must be introduced by a reflector between the front lens of the objective and the specimen.

The lower powers are not very suitable for this class of work; they have a relatively short free working distance, too short to permit of the introduction of a glass slip reflector, and also have marked curvature of field as compared with the large flat field given by the modern short-focus high-aperture micro-anastigmat lenses.

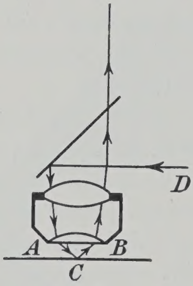


Fig. 101.

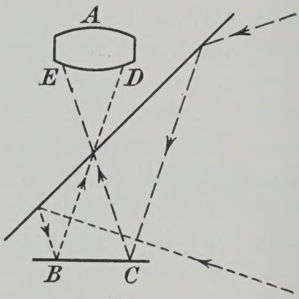


Fig. 102.

A lens of this type, with a focal length so short as $\frac{7}{8}$ inch and an aperture of F2, may be used in this way, giving good resolution and very satisfactory definition over a large flat field at magnifications of from 15 to 25 diameters. If the whole of the aperture is used, corresponding to an N.A. of about 0·22, the resolution obtainable is almost equivalent to that normally given by a 1-inch microscope objective as used for photographs at 50 to 100 diameters, and the field covered is much larger.

Lenses of this type are used without any ocular, the primary image being projected direct to the camera-screen ; the image-forming rays produce a sharply divergent cone of light from the back of the lens, too wide to be passed by an ordinary microscope body-tube or even by the usual size of reflecting prism which is mounted directly below the objective in microscopes of the inverted or 'Le Chatelier' type.

The microscope itself has been designed in the present instrument to accommodate a reflecting prism of extra large size, and the usual microscope body-tube is entirely removable, being replaced for this low-power work by a wide connection leading directly to the camera.

Illumination at low powers by a wide-angled vertical cone of rays is simple when relatively longer focus micro-anastigmats are used which give ample working distance between the lens front and the specimen. But when a very short focus lens is used, the incident beam is very likely to be obstructed by the stage itself. Considerable trouble has been taken to design a stage which will accommodate larger metal specimens and which will not obstruct the illumination to the shorter focus lenses. This particular difficulty has been avoided in some previous instruments by the use of longer focus lenses ; the results are not quite so satisfactory, as the same wide angular aperture is not attainable.

There are one or two other novel features in the microscope itself ; the fine-adjustment is of a new type and is particularly free from creep or backlash, the objective alone is moved by it ; the reflecting prism is completely removable for cleaning. The glass slip vertical illuminator used at medium and high magnifications is mounted in a very smoothly adjustable fitting, and is interchangeable with a prism type reflector if desired. The stage is mechanical, with a traverse of 30 mm. in either direction and has sufficient range of coarse-adjustment to focus a 3-inch micro-anastigmat. Each of three low-power lenses, micro-anastigmats of $\frac{7}{8}$-inch, $1\frac{1}{2}$-inch and 3-inch focus respectively, has its own glass plate reflector attached to it and mounted in gymbals. The microscope has two body-tubes at right angles, one for visual observation and the other leading to the camera ; the light from the objective is directed into one tube or the other by rotation of the reflecting prism. The draw-tubes are engraved with tube-length readings from 150 to 260 mm.

The attachable sub-stage illuminating equipment for use on transparent objects is of standard type and is complete. It includes a large Ahrens polarising prism and a fully rotating stage plate.

Illumination for medium and high-power work is by a system already described; the field lens in the system is interchangeable to meet the requirements of different tube-length settings.

The light-source is a 100-C.P. Pointolite lamp, and the whole illuminating train may be raised or lowered by a worm-gear movement and so brought into alignment with either the vertical illuminator in the microscope body, the low-power reflectors, or with the sub-stage mirror.

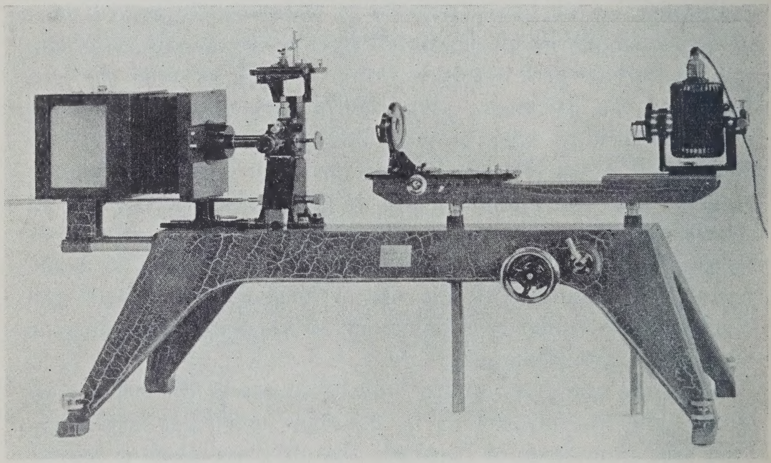


Fig. 103.—General view, as arranged for medium and high-power Metallography.

The raising motion works smoothly and without lateral shift, consequently the centring adjustments to the various condenser lenses, once properly set, need not afterwards be disturbed.

The camera itself is of half-plate size with variable extension, is very rigid, and of the reflex type; an exposing shutter is provided. The apparatus is assembled on a strong and rigid casting.

Figs. 103–105 show the apparatus set up for the various purposes described.

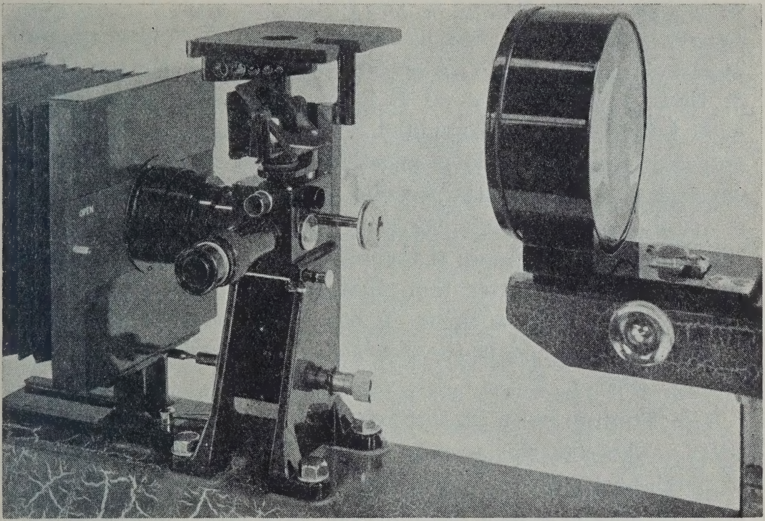


Fig. 104.—Showing illumination in low-power Metallography.

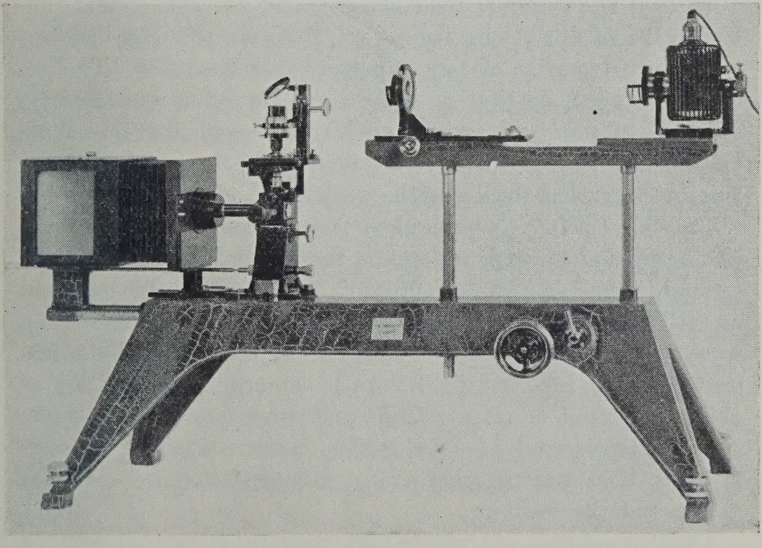


Fig. 105.—As arranged for Photo-micrography of Transparent Objects.

A new monochromatic objective for metallographic work, computed by Mr. Bracey of the British Scientific Instruments Research Association, has been used by Mr. Wrighton on the apparatus described. This objective is made by Messrs. R. & J. Beck, Ltd., and has an N.A. of 1·60 in the wave-length 4359 A for which it is computed. The focal length is 2·25 mm. and the immersion fluid is monobromide of naphthalene. No cover-glass is used as the working distance is too short, so it is mainly useful in metallography. The results obtained with this objective are fully up to theoretical expectations, the resolution is definitely better than that hitherto obtained by any objective using visible light. A description with photo-micrographs can be found in the *Journal of the Royal Microscopical Society*, 1933, p. 328.

The Photography of Tube- and Plate-cultures of Bacteria.—Although this process does not come within the scope of photo-micrography, it so often becomes necessary to obtain representations of such objects that no apology is offered for giving a description of the methods employed.

The apparatus required consists of an ordinary photographic camera with a suitable lens. The former may be any good camera of the stand or field pattern, and the lens should preferably be of fairly long focus—say from six to eight inches—and not necessarily of large aperture. It need not be of an expensive type, so that a modern anastigmat is unnecessary, although of course it may be used. All the photographs illustrating this section have been taken with a rapid rectilinear lens of six inches focal length, working at an aperture of F8.

The only point that requires special consideration in the photographing of such objects is the method of lighting. In general, tube- or plate-cultures of bacteria may be regarded as either opaque or transparent objects, so that illumination would be conducted as in microscopic objects, either by transmitted or by reflected light. As a compromise a method of oblique lighting is often useful, and where small semi-transparent colonies are lying on a gelatin or agar surface it is generally the only way to obtain any differentiation between the object and the medium.

The two methods of growing bacteria in tubes, usually referred to as stab or as streak cultures, in which the growth is either in the depth of the medium or on its surface, will require illuminating in different ways. The point to guard against is that, while the surface-growth or the growth in depth has to be properly illuminated, it must be done without any light being reflected from the internal or external walls of the glass tube itself, for such reflections interfere seriously with the proper delineation of the object. One method of avoiding reflections from the tube is to immerse it in a rectangular glass vessel which is filled with water, or a mixture of water and glycerine. With gelatin-cultures, water will usually be found sufficient, as its refractive index approximates to that of the gelatin ; but with agar or more highly refractive media, the water and glycerin combination is better. The exact proportions of the mixture are best determined by observing the effect on the appearance of the tube when more or less glycerin is added to the water ; but in general two parts of water to one part of glycerin will be found suitable. With the tubes placed in a glass cell in this manner, it is only necessary to throw an oblique beam from a fairly powerful source of light from a point either in front of or behind the tube. The camera is placed in a suitable position in front of the culture, and is extended so that it gives an image approximately the same size as the culture-tube itself. The lens will then require to be stopped down to F 11 or F 16 to obtain sufficient depth of focus. It may be necessary in order to obtain the best illumination to vary the angle of incidence of the light in a vertical direction so that the light is projected either from above the point of view or from below, or from one side, as required. Fig. 106 illustrates the relative position of the various parts.

The water-bath will require to be of fairly large size, so that there is considerable thickness of fluid all round the tube. One may be easily improvised in the manner already described for making up glass cells to contain the fluids for colour-filters (p. 211).

Between the source of light and the tube a condensing-lens may be placed, but it is advisable to arrange the lens so that a parallel, or approximately parallel, beam is projected on to the object. In photographing such objects in which there is considerable variation both of colour and general appearance, it is very difficult to give clearly defined instructions, and it is always necessary to do a certain amount of preliminary shifting of the illuminant before a satisfactory result is obtained.

The writers have set up a simple arrangement which may be used for photographing plate- and tube-cultures, and also many other objects of a similar character that may occur from time to time. Any ordinary object—such as a museum specimen—may be photographed with ease by its use (Fig. 106).

It consists essentially of a baseboard, say from three to four feet long and about two feet wide, on one end of which the illuminant A is fixed. The latter is supported in any suitable manner so that it has considerable range of movement in a vertical direction. At a distance of three or four inches in front of the illuminant is placed an opaque black screen B, and this screen must be varied in width to suit the particular object being photographed; in the case of tubes it may be, say, two inches broad, effectively blocking out any direct light that might pass from the illuminant A to the tube C placed in the position as shown in the diagram. On each side of the baseboard, and at about six to nine inches from its centre

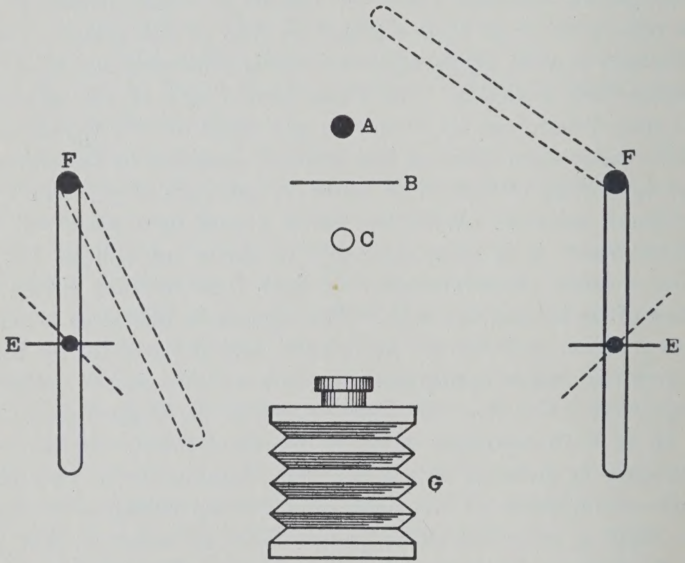


Fig. 106.—Arrangement of Camera and Illuminant for Photographing Tube- and Plate-culture of Bacteria.

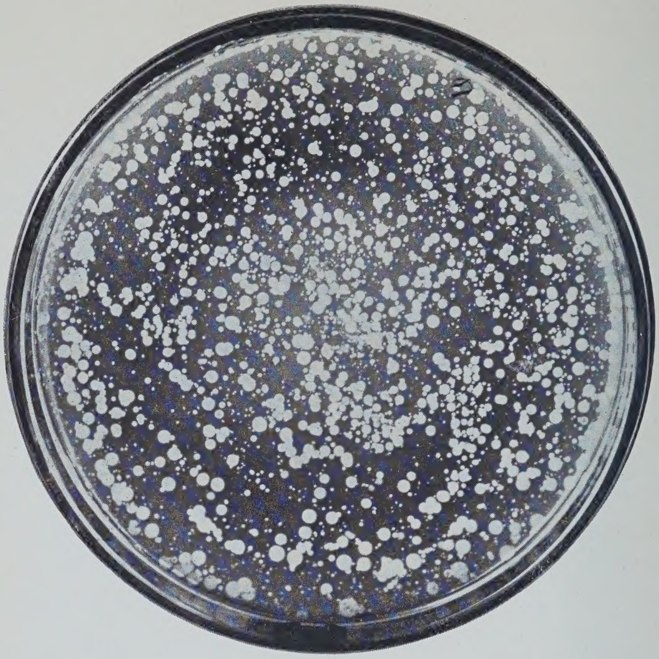


Fig. 1.—Plate Culture of Bacteria (Opaque Colonies).

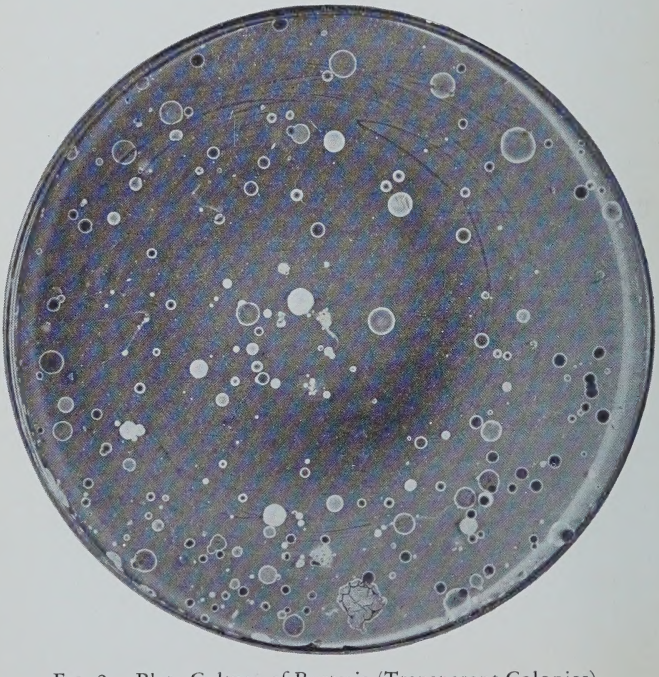


Fig. 2.—Plate Culture of Bacteria (Transparent Colonies).

vertical brass rods FF are fixed. On each vertical rod F a horizontal rod is placed which is free to move on the rod F and can be clamped in any position. Mirrors are supported on the horizontal rod as shown at E and can be rotated on their points of support as shown, and inclined at any angle. It is evident therefore that by altering the position of the mirrors or by varying the position of the supporting-rods, the light coming from the illuminant A may be reflected at any angle on to either or both sides of the object; or the illumination may be by reflected or transmitted light. If the object is to be shown on a dark ground no direct light reaches the object from the illuminant owing to the opaque screen which is interposed. A ground-glass screen may be substituted for the opaque screen should directly transmitted light be wanted. This arrangement has the advantage that while the angle of the light may be varied at will, the illumination if required may be perfectly even from both sides; that is, if the position of the mirror in relation to the source of light is exactly the same on either side, even illumination will be obtained. The appliance may therefore be used for copying diagrams, which are simply placed on the opaque screen and the light reflected on to them. Exactly the same conditions apply in photographing plate-cultures, as these have either opaque or transparent colonies on them. The opaque ones, however, should be illuminated by reflecting the light on to their surface (Plate X, Fig. 1); whereas with those having transparent colonies (Plate X, Fig. 2) the beam is projected from behind, but at an oblique angle so that the colonies themselves show up light on a dark background.

Plate XI gives examples of photographs of tube-cultivations.

a. Coagulation of Milk by Bacillus coli communis. Photographed by reflected light, and with the illuminant placed well below the level of the tube so that reflection from the tube itself was thrown upwards. There is, however, some slight evidence of tube-reflection, a streak showing just near the centre, but it is hardly sufficient to interfere with the result. The culture-tube was not immersed in any tank and shows that it is possible to diminish tube-reflections by suitable adjustment of the light.

b. Bacillus coli communis, Gelatin Shake-culture showing gas-production. Photographed by directly transmitted light. Instead of the opaque screen the ground-glass screen was interposed between the source of light and the tube.

c. Klebs-Löffler or Diphtheria Bacillus on blood serum. Oblique culture with surface colonies, showing very little differentiation so far as the colour is concerned. The colonies, however, have a glistening highly reflecting surface, and by careful arrangement of the light it is possible to bring them out showing brightly on the less bright ground. No water-bath was used in this case, but the result was obtained by reflecting the light from a point well above the level of the tube.

d. Bacillus tuberculosis on agar-agar. Photograph obtained by immersing tube in a water-glycerin bath ; illumination, by reflected light. The outline of the tube is barely seen.

e. Bacillus of Tetanus, Stab-culture in sugar-agar. A rather dense agar-tube with growth entirely in depth and at centre of tube. A white screen was placed in the position usually occupied by the opaque black screen, and light was reflected from the mirror on to the surface of the tube. The illumination was well above the level of the tube, so that reflections were thrown downwards.

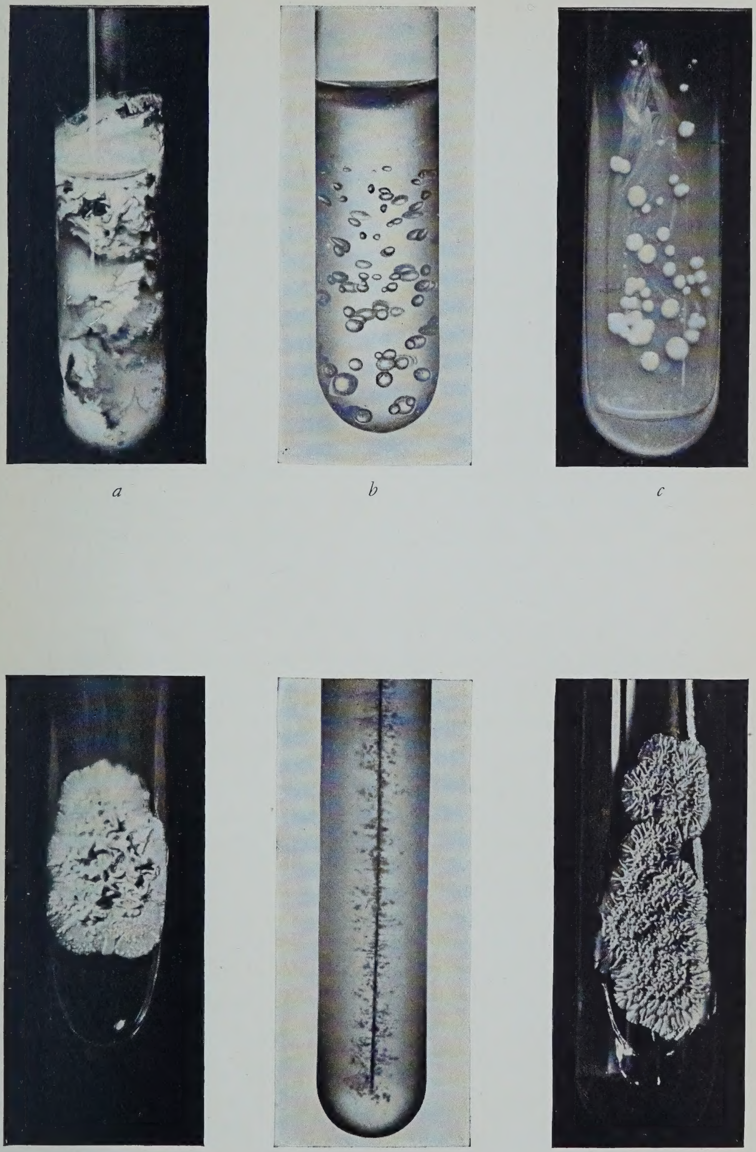
f. Bacillus tuberculosis on agar-agar. Very oblique illumination of a surface-growth on agar. The light was placed at a very acute angle to the surface of the agar in the tube, so that the greatest possible contrast was obtained to show up the convolutions in the growth itself.

The exposure required in such cases must of course be dependent on the illuminant. A plate of medium rapidity should be used, so that the greatest contrast is obtained.

It should be remembered, when using any source of light of high power, that gelatin-cultures will melt with a relatively small rise of temperature ; hence the tubes should be well shielded from any direct light, and if they are contained in a water-bath should not be left long enough for any rise of temperature of the water to take place. In summer even a rise of four or five degrees will be quite sufficient to melt the contents of the tube. If the tubes are contained in a water-bath, the bath itself as well as the outside of the tube should be perfectly clean, as any impurities will show up only too clearly in the photographs.

A condition that sometimes does arise and that gives a great

PLATE XI



a *b* *c*

d *e* *f*

Tube Cultures of Bacteria.

deal of trouble is due to the condensation of water-vapour on the inside of the culture-tube itself. When preparations are permanently sealed the difficulty is a considerable one. If such condensation appears, it is generally due to the tube being taken for photographing into a room which is of lower temperature than that of the incubator in which the growth has been allowed to develop. In certain cases it may be possible to remove the wool plug and by means of a small bit of cotton-wool held in long forceps to wipe the condensed vapour off the inside of the tube. This must be done with the utmost care to avoid disturbance of surface-growth, and in dealing with pathogenic organisms the usual laboratory precautions should be taken. A better way, where the cultures cannot be interfered with, is to place the tube for a short time in a box containing some calcium chloride. In the course of from two to three hours this will usually absorb sufficient moisture to cause evaporation of any free water-vapour in the tube which might otherwise be sufficient to prevent a photograph being taken.

To reproduce an object in its natural size, the distance of the ground-glass screen of the camera from the lens must be double the focal length of the lens (see Appendix IV). This distance may be determined with sufficient accuracy by taking the focal length of the lens—which is usually marked on the mount—and measuring the distance from the iris-diaphragm of the lens to the ground-glass focussing-screen. Obviously a camera with sufficient extension will be required, but most half-plate field cameras are long enough. The object must then be placed at approximately the same distance from the lens-diaphragm. When the image is required to be larger than the original, the lens should be reversed in the camera-front; that is, the back combination of the lens should be placed towards the object, and the image projected with the front of the lens facing the ground-glass screen. In this case no definite instructions can be given as to distance of object or extension of camera, and these must be determined by experiment to suit the particular case. Where symmetrical lenses are used—such as a Zeiss double Protar—moderate magnification, up to ten or fifteen diameters, may be obtained with the lens in its normal position in the camera-front.

The appliances already described for dealing with culture-tubes and plates may also be used for pathological or similar specimens. In this case, the object may be supported in the position C, and illumination effected as with culture-tubes in which the growth is on the surface. It is sometimes difficult, however, with such objects to support them conveniently in a vertical position, and it is therefore an advantage to have all the appliances described fastened to the baseboard, so that it may be supported in the vertical position. The object may then be laid on a glass plate, and illumination effected from above or below as required. With pathological specimens a source of trouble is that they are often in a wet condition, and therefore some parts have a glistening appearance. In such cases they should, if possible, be surface-dried by immersing them in alcohol for a short period. Where this cannot be done, it is often permissible to dust lightly over their surface some fine granular powder, such as powdered starch. This does away with the reflecting surface, and may be washed off again immediately the photograph has been taken, without any harm resulting to the specimen.

Many pathological specimens can be placed in any suitable dish and completely immersed in fluid to overcome the same difficulty. There are then no reflections from the surfaces of the material and colour differences are satisfactorily rendered in the photograph. It is important to see that the fluid is clean, free from air-bubbles and with no particles suspended in it. As most museum specimens are kept in fluid for preservation this method has much to recommend it.

Stereoscopic Photo-micrography.—The production of stereoscopic photo-micrographs is confined almost entirely to low-power work, and the best results are to be obtained with objectives of not less than one-inch focus. The process must not be confused with ordinary binocular microscopic vision. It consists essentially of obtaining two photographs side by side on one plate from different points of view in relation to the object, or by means of different zones of the objective. It is also dependent on getting more than one plane of the object in focus; in fact, as it depends essentially on viewing the object in three dimensions, the focal length of the objective must be considerable. In addition, means are generally taken to restrict the aperture of the objective as far as practicable and thus to obtain the utmost penetration.

The apparatus need not differ from any already described, providing that the dark-slide is large enough to enable two photographs to be taken on the same plate. There are three methods at present in general use. The simplest is to cover one half of the back combination of the objective by means of a stop; an appliance to do this consists of a short fitting which screws in between the objective and the nosepiece of the microscope. A slot is cut in this fitting, into which is dropped a blackened brass slip, so that exactly one half of the back combination of the objective is covered. One negative is taken through the uncovered half, and then the stop is transferred to the opposite side of the aperture of the objective and another negative is again taken on the same plate. The two pictures are therefore side by side or they can be taken on separate plates. Prints from these negatives, when suitably mounted, yield a satisfactory stereoscopic effect.

Instead of covering and uncovering half of the objective, a better method is to place in the same position stops with circular openings. The advantage of this method over the one just described is, that owing to the shape of the diffusion-discs in those parts of the object which are not in exact focus being dependent on the shape of the stop at the back of the objective, such diffusion-discs are at their best with circular openings. There is the further advantage that both the size and separation of the openings may be varied to suit the particular objects, or to increase or decrease the amount of desirable penetration. The procedure is precisely the same as before, the stop being placed at one side of the objective and then removed to the opposite side to take the succeeding photograph. It is obviously not a matter of moment whether, in relation to the position of the microscope, the stops are side by side or above and below; this will depend simply on the position of the plate in relation to the apparatus, and on the orientation of the object.

The size of the circular openings may vary between $2\frac{1}{2}$ mm. and 4 mm., and the inner edge of the aperture will not generally require to be more than 1 mm. from the optic axis of the objective. The size of stop should be the largest possible to give the necessary penetration and to bring out detail sharply in the picture. The inner edge of the aperture should be as near the centre of the objective as will give the necessary stereoscopic effect. These circular stops are carried in a suitable fitting at the back of the objective, and may be obtained from Messrs. Baker of High Holborn. A repeating back may be used instead of an ordinary dark-slide. The advantage is that when prints are made from the negatives they are in their proper position relatively to one another for use in a stereoscope—that is, they do not require cutting and remounting. It is important, whichever method is adopted, to see that the apertures are in exact alignment when changed from side to side; otherwise the resulting pictures will not superimpose to give the necessary stereoscopic effect.

A third method of obtaining the same result is by shifting the objective or the object by a definite amount from side to side. The principle involved is that the inter-ocular distance, normally 62 mm., must be divided by the required magnification to give the angular separation through which the objective or the object must be moved. Thus for 32 diameters the separation required would be nearly 2 mm. In practice it is found advisable to shift the object by the required amount rather than the objective. The degree of necessary separation may be determined by ruling two lines on the focussing-screen at a suitable distance apart, and then moving the object from side to side so that the centre of the field of view coincides with the lines that are ruled on the screen. The exposures are made in succession as in taking ordinary photographs. The objectives most suitable are those of the Zeiss planar type, as their field is flat and their aperture is such that the maximum penetration is obtained.

The majority of suitable objects are best illuminated by reflected, rather than by transmitted, light. Any source of light may be used, and exposure will vary within the same limits as in ordinary photo-micrographic work. The illuminating beam may conveniently be a convergent one. There are no unusual practical difficulties to be encountered in this class of work. It is essential that the two pictures receive exactly the same effective exposure, and that in development the same treatment is given to each. If the two pictures are on the same plate the latter requirement would be complied with in the ordinary course. Where succeeding photographs are taken, a high-power source of light, like the electric arc, should not be used, as there is often variation in the constitution of the carbons, and the actinic power is therefore not exactly the same for a given period. It is also more difficult with high-power illuminants to give precisely the same exposure to each; a very slight variation would cause an appreciable difference in the two negatives. Practically, therefore, it is better to use a source of light which is constant, and which entails an exposure of moderate length. The methods so far described are simple and do not involve the use of expensive appliances. They do not, however, give such optically perfect results as the Greenough Binocular microscope, in which two accurately paired objectives are used. This instrument is well known and need not be described here in detail. Messrs. Carl Zeiss make a camera attachment for their Greenough Binocular which replaces the body-tube of the microscope and enables two photographs to be obtained with one exposure (Fig. 107). The body of the camera resembles a truncated pyramid, and is divided inside by a longitudinal partition. At the larger end a frame is carried on which the two focussing-screens rest and is replaced by the dark-slide when the exposure is made. The paired objectives are carried on a slide on the camera-front. A time and instantaneous shutter is provided. The dark-slide carries two plates which are set at an angle to one another so

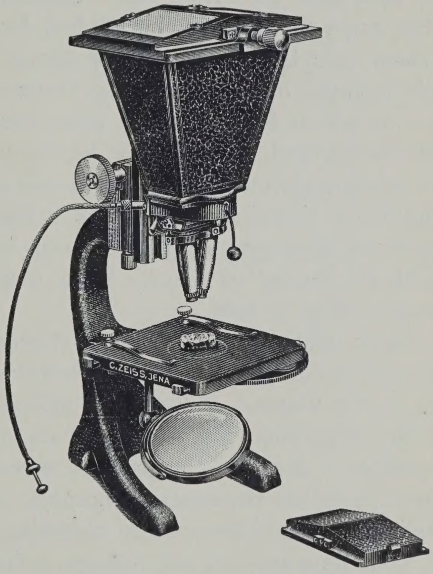


Fig. 107.—Druner Stereo-Camera (C. Zeiss.)

that each plate is at right angles to the axis of its own objective. The manipulation is simple, with opaque objects particularly, as the object only needs to be illuminated in such a way as to show it to advantage. With transparent objects it is necessary to exercise care to see that the illumination on each side is equal, a state of affairs that may easily be departed from. This is most easily achieved by illuminating each objective with an independent reflector, either two mirrors or two white surfaces. A single light source is used and the reflectors are placed so that light goes from each centrally through each objective. The same method can be applied for oblique illumination, in which case the object can be shown on a dark ground. All photographs obtained with the Greenough Binocular are of low power and a few trials will soon demonstrate the possibilities of the method. These may be viewed without a stereoscope if accommodation of the eyes is relaxed (that is, an object at infinity is looked at steadily for a few moments) and then the pairs of photographs placed about ten inches from the eyes without change of focus. With a little practice the stereoscopic effect is seen.

Many methods of obtaining stereoscopic images have been described during the last fifty years or more and may be referred to in the *Journal of the Royal Microscopical Society.*

The diagrams (Fig. 108 A, B and C) indicate the principal methods. A shows the path of rays when two objectives are used as in the Greenough binocular. B when a stop is placed behind the objective or the objective is displaced laterally.

C shows a method not hitherto referred to here in which the object slide is tilted to the necessary angle on each side of the optic axis and a photograph taken in each position.

The method of mounting stereoscopic prints needs some attention. With the first two methods described the pair of prints, which we assumed to be from one plate in an ordinary printing-frame, are separated. To mount up for viewing, these two prints are reversed in position, the left-hand one being placed on the right side and the right-hand print on the left. The distance between the centres of the mounted prints should be between 60 and 65 mm. Transposing printing-frames can be obtained by the use of which the necessity of cutting the prints is obviated.

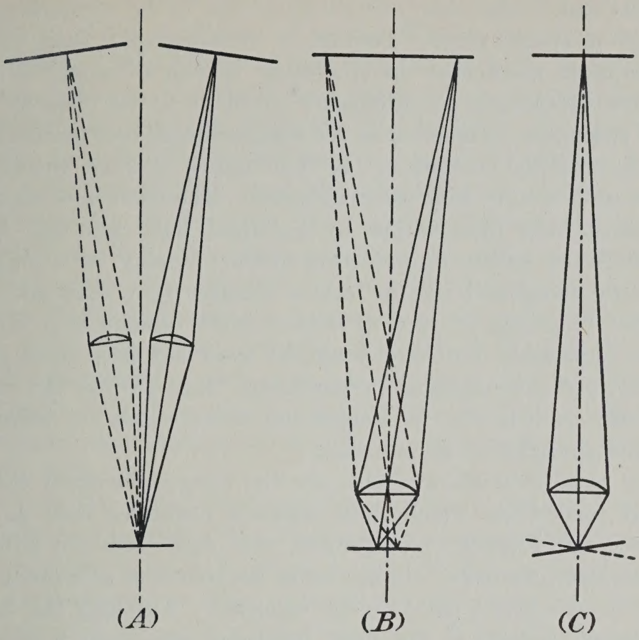


FIG. 108.

The mounting of the Greenough Binocular prints is different. The prints are not reversed in position but must be rotated in the plane of the paper through an angle of 180°.

The Production of Lantern-slides in Colour.—The production of lantern-slides in colour has now become such a recognised method, and is, under certain conditions, of such utility, that a short description of the principal available methods should be given. It is, however, only a few objects to which the process is applicable or is indeed necessary. The majority of stained objects, although it improves their appearance from a pictorial point of view to represent them in colour, are shown equally well by ordinary photography. Staining methods are themselves arbitrary, and are only employed in order to differentiate structure; their differences therefore are usually as well shown in monochrome as by any colour method. Coloured lantern-slides of necessity involve more work in their production; the results from a scientific point of view are not so exact, and they are therefore not to be recommended except in special cases. Perhaps in metallography their use is appreciated more than in any other branch of work, as the surface iridescence, or difference in colour to be observed in such specimens, is inherent in the specimen and does not depend on any artificial coloration. In that branch of work, therefore, some good results have been obtained. The colours of objects resulting from illumination by polarised light are also best shown by a colour reproduction method; many such objects are only recognised by the colour changes that they set up. Correct rendering of such objects is often difficult and sometimes impossible, but very beautiful work has been done particularly of mineralogical preparations. Exposure is the chief difficulty, as it is often prolonged and varies widely for different polarising angles of illumination.

The chief methods available are the Sanger Shepherd three-colour process (in which three separate negatives have to be taken), the Lumière Autochrome, the Agfa and the Finlay colour-plate processes. In the latter the colour effect is obtained directly on a single plate by one exposure. Although the first-mentioned process is the most laborious one, from a photo-micrographic point of view the results are superior to any other, as the image is absolutely grainless and is very transparent.

The necessary materials for carrying out the first process are to be obtained from Messrs. E.S.S. Colour Filter Co., London, and full detailed instructions for the work are supplied with the outfit. The method consists essentially in obtaining three separate negatives on panchromatic plates, one each through a red, green, and blue-violet filter. The exposures will be dependent on the source of light, but need not be of great length. The time of exposure will not be the same for each screen, but will vary according to the colour-sensitiveness of the plate and the spectroscopic value of the source of light. As an example, with a given illuminant the exposure might be 20 seconds through the red screen, 100 seconds through the green screen, and 25 seconds through the blue-violet screen. It is important to ensure that the relative exposures are correct; a small error in the absolute exposure is not serious provided that the relation is maintained.

The most suitable illuminants are those which approximate most nearly to daylight. A source of light, therefore, such as a mercury-vapour lamp is not suitable since its spectroscopic value differs so materially from any other. The electric arc, the half-watt lamp or Pointolite may be used. The arc lamp, owing to variability in the composition of the carbons, is not the most satisfactory, and the possibility of change in the actinic value of the light with varying voltages and current density, disturbs the balance between the three negatives.

The plates are exposed successively through the coloured filters mentioned, and are developed in the same solution for the same time so as to obtain as nearly as possible the same type of negative in each case. As the plate used must be sensitive to the whole visible spectrum, the dark-room illumination must be such that plates of this sensitiveness may be safely dealt with.

Up to this point—the production of the negatives—the process is one that differs in no respect from ordinary photography. From each of these negatives a positive has to be obtained. From the negative taken through the red screen an ordinary lantern-slide on glass is made, and this is toned to a suitable blue colour with the solution supplied for the purpose. From each of the remaining two negatives a positive in bichromated gelatin is made on celluloid which may be procured ready sensitised from the makers, and should be dealt with in accordance with their instructions. They are printed from the negative in exactly the same way as an ordinary lantern-slide, except that the unsensitised celluloid surface is placed in contact with the film-side of the negative in a printing-frame and exposed to a suitable source of light. The exposure must be determined by an actinometer, as it is important that this be correct ; but in any case some preliminary experiments may be necessary before the exact duration is arrived at. On removal from the printing-frame some indication of the image is seen, and the films must now be placed in water at a temperature of 90° to 105° F., until the unaffected portion is dissolved out. This may be carried out in the dark-room, by orange or bright-yellow light. If the image does not appear in relief in a few minutes but remains undissolved, the temperature of the water may be raised until the desired effect is obtained. When development is complete, the films are placed in a weak solution of hyposulphite of soda—say 1 in 40—until all the bromide of silver not acted on is dissolved out, after which they are washed. These two films now have to be stained with the dyeing-solution supplied by the makers, and it is on the exact degree to which this process is carried that success depends. Possibly the best method is to over-stain each of the impressions, and then to immerse them in water and gradually dissolve out the stain until sufficient remains. This method allows of more precise control than an endeavour to remove the films from the stain at the exact moment when they have been treated for a sufficient length of time. A point to be borne in mind is that unless the exposure is strictly correct for each impression, there is a possibility of the images differing somewhat in size, so that on binding them together it is impossible to place them in exact register. After drying, the films are varnished, and have then to be fastened together by a suitable cement, so that they exactly overlie one another. The instructions on this point, as given by the makers, are sufficiently clear. To obtain the greatest transparency, the films should be cemented together with Canada balsam, and it is important to ensure that no extraneous matter and no air-bubbles are allowed to get in. The slide is then bound up, and if a mask is desired it is preferably placed on the outside of the slide. If the whole process has been efficiently carried out, a reproduction in colour results which is of perfect transparency and colour-rendering.

The Lumière Autochrome method is essentially different in principle and practice. Instead of taking a separate negative for each of the three primary colour records, the whole colour effect is obtained at one operation and on one plate or film. Further, no negative is taken, but the direct colour positive is given by the plate that is exposed in the camera. This is accomplished by coating the plate with a series of filters, which consists of minute dyed grains of starch. Over this the emulsion is spread so that the exposure takes place through the combined colour-screen, and is visible on development of the plate. The instructions given by the makers are so complete that it would not appear necessary to repeat them in detail. The points that require special consideration in photo-micrography are the source of light and the yellow subsidiary screen through which the photograph is taken. It happens that with daylight, or with any other source of light, the plates as prepared are still too sensitive to the blue end of the spectrum, so that a yellow-glass screen has to be interposed between the source of light and the object, to cut out the unnecessary portion of the light. When daylight is used these yellow screens as supplied by the makers, are perfectly efficient; but with artificial light-sources either an additional screen or one specially prepared becomes necessary. The source of light should approximate as nearly as possible to daylight. If an ordinary petroleum lamp is used excellent results may be obtained without using any screen; in fact, some of the best results that have passed through the writers' hands have been arrived at by this means.

The Autochrome plate is of fine grain, but, fine as it is, lantern-slides made by this process still show some evidence of granularity when projected on the screen. In the Autochrome plates the starch-grains of a particular colour tend to form themselves into groups, the actual advantage of the Autochrome method is from this point of view not so great as might be expected from theoretical considerations. The Finlay, Agfa and Spicer-Dufay processes give somewhat greater transparency; but with either method, when projecting the colour positives, an illuminant must be used of high power, otherwise a brilliant image on the screen cannot be obtained.

The Finlay is also a screen plate process, the colours being obtained by means of a glass plate which is covered with minute squares of red, green and blue-violet colour. But this process is a duplicating one, so that any number of coloured transparencies may be obtained from each negative. Only one exposure in the camera is necessary to produce a negative from which any number of coloured transparencies, all identically the same, can be obtained. Only ordinary photographic knowledge is needed, and anyone who is accustomed to develop his own negatives will find no difficulty in making one for colour. The articles required are the taking screen, the compensating filter, the panchromatic negative plate, the special transparency plate, and the viewing screen.

The panchromatic plate and the colour filter are essential, as these plates and filters have been very carefully made to work correctly with the taking screen.

The taking screen is a glass plate covered with a regular pattern of minute squares of red, green and blue-violet. This screen is just as important a part of the camera as the lens, and if care be taken of it, it will be capable of taking hundreds of negatives. The screen-plate and sensitive plate are placed in the dark-slide face to face, with the glass side of the screen-plate towards the lens. It is of the utmost importance to have good contact between the taking screen and negative plate, and this can only be obtained by having efficient springs in the dark-slide.

The viewing screens are of the same pattern as the taking screen, and are used for binding up with the transparencies to produce the required colours. These must be kept separate from the taking screen, as although they look the same, they are not interchangeable.

As the registration of the viewing screen with the transparency plate is the only part of the process which presents any difficulty at first, the process is fully described by the makers in their booklet, to which reference should be made for full working instructions.

The foregoing colour processes yield pictures on glass only, and are therefore mainly suitable for projection purposes. The only method of producing colour prints available for the ordinary worker is the Autotype Trichrome Carbro Process. This process is the same in principle as the Sanger Shepherd method described, except that from the three negatives obtained, prints are made on carbon tissue supplied by the makers. Each of these is in a selected colour and when the three prints are superimposed on a paper support in exact register, give satisfactory colour rendering. In common with all three colour processes which depend on the perfect combination of complementary colours, good results are only to be obtained by carefully following the instructions of the makers. A booklet is published by the Autotype Co. in which full working instructions are given. Under favourable conditions and with suitable objects, excellent results can be obtained, but considerable practice appears to be necessary.

X-Ray Photo-micrographs.—It is recognised that there is no essential difference between X-rays and ordinary light. Except that their wave-length is different, they are manifestations of the same form of energy. If therefore some means could be found of utilising these much shorter wave-lengths, the progress in microscopic methods would be enormous.

The production of photo-micrographs by means of X-rays is not yet possible in the same sense that they can be produced by ordinary light. Until an efficient method of reflecting or refracting X-rays is discovered, no substantial progress is to be expected in this direction. No artificially polished surface is suitable and the only substances available are those which have natural cleavage planes, e.g. mica and selenite. Even then the loss of energy is enormous and the difficulty of obtaining suitably curved surfaces has not yet been overcome. The only practical method at present is that described in the *Journal of the Royal Microscopical Society* (1915, pp. 1–7).

The technique is quite simple in principle. It consists of the production of an ordinary X-ray photograph, on a fine-grained photographic plate, of a suitable microscopic object in the actual size of that object; any subsequent enlargement of the image is obtained by photographic means. The attainable magnification is therefore mainly dependent on the grain of the plate, although the original image may be of extraordinarily fine definition owing to the short wave-length of X-rays. The simple apparatus used is seen in section in Fig. 109. A is a small light-tight box carrying the object and the photographic plate. The object is supported on a piece of the thinnest obtainable black paper and the plate laid on it film side downwards. This box rotates on the centre C, the axis of which passes through the plane of the object. D is a quadrant showing the deflection of the plane of the plate from the normal. This movement is provided to enable photographs

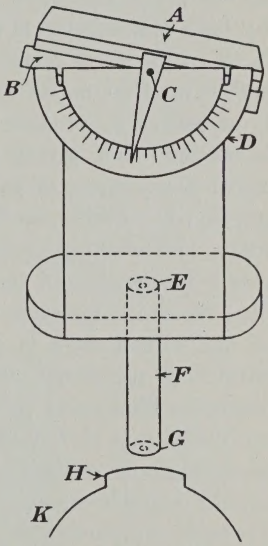


FIG. 109.—Plate-holder for X-ray photographs.

to be taken on either side of the normal so that a better idea of structure may be obtained. It is of great value in photographing such objects as foraminifera, examples of which are shown, or any similar objects having successive layers of structural details. Not only can the form of these be seen but the distances between the various parts may be measured. The image can in fact be obtained in any azimuth, the only practical precaution necessary being that the object must be at the centre of the plate as well as in the plane of rotation. A lead tube F is supported above the X-ray tube K, and each end of the tube is closed by lead diaphragms E and G having small central holes. The X-ray tube itself must be one that gives a preponderance of soft rays, that is, its exhaustion must not be any higher than is necessary to produce X-rays at all. A small coil is all that is needed; one giving a spark of four inches is sufficient. A small X-ray tube, of the type used in the early days of radiography, is suitable, as the actual energy output needed is very little. Exhaustion should be such that the characteristic yellow-green fluorescence of the tube is seen accompanied by a definite blue stream impinging on to the anticathode. If an emission of the softest rays is desired, a tube having a lithium glass window is necessary, and can be obtained commercially. The examples illustrated (Plate XVII) were taken with such a tube on Wellington slow lantern plates. The method requires some knowledge of ordinary X-ray work, and the earliest efforts should be made on such objects as leaves or thin stems of plants. There is no particular difficulty in the process except that a good deal of experimental work is necessary to enable correctly exposed plates to be obtained.

Micro-cinematography.—The combination of the microscope and cinematograph was attempted in the early days of cinematography with considerable success. Pictures of living objects, particularly pond life, were shown by Mr. F. Martin Duncan about thirty years ago at the old Alhambra in London to appreciative audiences, so such work can hardly be regarded as of recent birth. The difficulties then as now were mainly associated with the biological material itself. The effect of strong light and the elimination of harmful heat rays was the principal trouble, but the improvement in the sensitivity of films has now reduced the necessary exposure. The principle of the method is simple. Providing that the moving objects are well shown and are illuminated by a sufficiently powerful light, there is no special difficulty. The apparatus shown in Fig. 110 is typical and has been used by the writers in conjunction with the illuminating system shown in Plate IV. Beyond the microscope and its essential accessories a cinematographic camera is placed to receive the projected image. The only other special appliance is an eye-piece which enables the observer to watch and continually focus the object whilst exposure is proceeding. The object is seen and focussed by the side observing tube and the camera is placed at such a distance from the microscope that the image is in focus on the film at the same time. The focussing eye-piece is seen in plan in Fig. 111. A is the projection ocular on the microscope which throws the image directly on to the film in the cine-camera in the direction B. At the position C two similar right-angled glass prisms are supported on a pivot so that they may be rotated on a

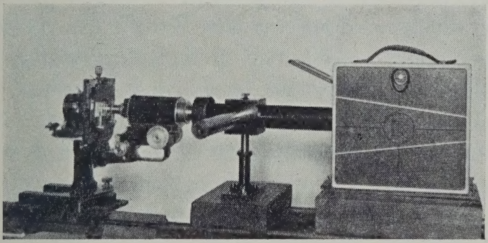


Fig. 110.

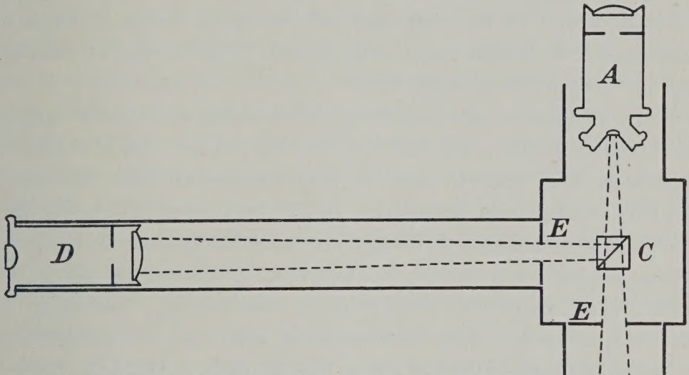


Fig. 111.

vertical axis for adjustment purposes. These two prisms are cemented together with a thin film of cedar-wood oil in the position as shown. The beam from the microscope therefore passes through these two prisms, but owing to the slight difference in refractive index between the cedar-wood oil and the glass, some small portion of the light is reflected into the observing ocular D. As the source of light used is an arc-light, the amount of light thrown through the side-tube is enough for observation and for focussing, but does not result in any appreciable decrease in the intensity of the main beam. Stops are placed at *E E* which strictly limit the beam to that necessary, and obviate internal reflections.

Any well-made cine-camera can be used for such work together with either 9 mm., 16 mm., or 35 mm. film. In the writers' apparatus the camera used is that known as Cine-Kodak A, and this is shown in Fig. 110. It can be driven either by hand or by electromotor. Although it is actually an early model and is largely superseded for ordinary purposes by the clockwork-driven cameras, it has every movement necessary for microscopic work. It is substantially made and can sometimes be purchased second-hand for a few pounds. The lens may be removed, but this must be done by the Kodak Co. as it is mounted inside the camera-body and is difficult to get at. The whole apparatus must be supported on a rigid base and the camera itself tightly screwed down to prevent vibration. It is much more important to secure complete rigidity than even in ordinary photo-micrography. The observing tube must be supported separately from the camera and microscope, the only connection is a light-tight joint which does not touch either. This method of connection has already been described. The illumination of the object and the adjustment of the microscope is secured by reference to an image projected on to a white screen placed at any convenient distance. This ensures accuracy of alignment and centration. The camera and the observing unit are then introduced and the prisms adjusted by the small knob shown in Fig. 110 so that the image can be observed and focussed in the side-tube. The object being seen, the camera movement can be started and a film exposed. The number of pictures taken in any given time is, of course, dependent on the object, but the time interval can be varied within any limits with this camera. If hand cranking is adopted, it is a convenience, if not a necessity, for one worker to observe and another to operate the driving mechanism of the camera. With this in view, the apparatus shown has been arranged so that the observer is on the side of the bench opposite to the camera control. It will be seen that the microscope is in the horizontal position. For the photography of living objects it is customary to have the apparatus vertical, in order to avoid in the preparation any flow or movement due to gravity. If the usual thick preparations are used, this is an important point, but far better results will be obtained by making the layer containing the object as thin as possible without actually restraining or compressing it. Under these conditions, the need for a vertical microscope will disappear. Hanging drop preparations are unsatisfactory for photo-micrography of any sort. Should a vertical microscope be unavoidable, the most satisfactory procedure is to support the camera on a stiff framework over the microscope body-tube.

Any small fixed focus cine-camera can be applied, the lens remaining on the camera and being set at infinity, but it is better to remove the ordinary photographic lens if this can be done. As the resulting image is of necessity of small size, absolute sharpness is essential. This is dependent on perfect adjustment of both the illuminating and the observing systems of the microscope; strictly accurate setting up of the apparatus is essential, as in all branches of photo-micrography.

Photo-micrography with Infra-red Light.—The use of infra-red rays in photography is not new, but recently the necessary materials have become available commercially at a reasonable price. Its application to photo-micrography is recent and for certain classes of objects which are opaque to visible light it has proved to be of considerable value. The number of such objects is not large, but they do represent a class that has been difficult and sometimes impossible to photograph by ordinary means. Entomological preparations are often particularly troublesome as visible light will not penetrate the dense chitinous portions without hopelessly over exposing the thin transparent appendages that are usually present. The usual method with such objects has been to photograph them with a red or deep orange screen, using plates sensitive to these colours, but even then some objects do not yield a satisfactory negative. It is not intended here to give full working details of the process of infra-red photography, but rather to draw attention to its possibilities. The worker is advised to study the small but extremely lucid book containing an account of the technique involved, written by Dr. S. O. Rawling of the Ilford Research Laboratories and published by Blackie & Sons, Ltd. All the necessary materials may be obtained from Messrs. Ilford, Ltd., but Dr. Rawling has not confined himself to a description of their products. The essentials are an illuminant emitting the necessary infra-red rays over a range from about 7600 to 10,000 A°. An ordinary electric lamp or a Pointolite lamp together with a screen supplied by the Ilford Co. will provide such a source of energy. The camera should preferably be provided with a metal or stout ebonite dark-slide and both it and the camera bellows should be covered both internally and externally with a good layer of dead black. Most woods used for cameras are not sufficiently opaque, the safest materials are indicated in Dr. Rawling's book. The dark-room should be illuminated with a suitable safe light. One of the yellow or yellow-green screens suitable for ordinary blue sensitive plates may be combined with a blue glass selected for its power to absorb infra-red ; the result is a very efficient dark-room illuminant for use with most available plates, although in one case at least the plates must be handled in total darkness. Plates are usually packed in black paper which is quite opaque to infra-red rays and this material may be used for covering plates during manipulation. An Ilford Infra-Red Safelight can be obtained which gives a green illumination in the dark-room, similar to the green safe light commonly used for panchromatic plates, and in this light plates can be handled with safety.

Development presents no difficulty, particulars are given by the makers and these should be followed. The whole process is simple to those who have knowledge of ordinary photo-micrography. The image may be focussed on the plain glass focussing-screen, and so far as our experience goes with well-corrected objectives the difference between the visual and actinic focus is negligible. Examples are shown on Plate XXII, Figs. 1 and 2.

The difference between the two images is obvious. Whereas in ordinary light there are considerable differences in gradation, the use of infra-red light tends to flatten out the whole, penetration is almost the same in the dense as in the transparent portions. There is evident loss in resolving power as the result of using light of long wave-length, but in such objects this is not a matter of primary importance. There is no doubt that in some other branches of microscopy this method will prove to be of even greater value.

CHAPTER XII

PHOTO-MICROGRAPHY BY MEANS OF ULTRA-VIOLET LIGHT

Since the researches of Abbe which resulted in the production of apochromatic objectives, no increased resolution in the microscope has been achieved except by the use of ultra-violet light. The shortest wave-length that it is convenient to use for visual purposes is in the region of about 520 $\mu\mu$, but as it is now possible to utilise ultra-violet radiations of a wave-length of 275 $\mu\mu$ or less, resolution is thereby at least doubled.

To take full advantage of this, Messrs. Zeiss produced an apparatus about 1904, designed by Dr. A. Köhler, in which the source of light lies in the region of the ultra-violet; and the wave-length of that light is such that the resolving-power of the optical system is double that of any objective used for visual work.

It is well known that many organic substances are opaque, or partially opaque, to ultra-violet light, although under ordinary conditions of illumination no difference in transparency of the component parts is to be discerned. It therefore follows that by using such light for illuminating purposes structure may be brought out, which by any other method of illumination, or by differential staining, is invisible. Although an object may appear colourless by white light, it would in fact appear to have colour differences due to differences of absorption, if illuminated by ultra-violet light. It is obvious that no direct observation of the image can take place, and that photography is the only means by which a record can be obtained of the structure of such objects.

The modern outfit for ultra-violet photo-micrography consists of the following components:

1. An electrical transformer which will give a high-tension discharge between suitable metallic electrodes.
2. An adjustable spark-gap to hold the electrodes so that the length of spark and its position may be kept constant.
3. A quartz spectroscopic system, consisting of a collecting-lens and two quartz prisms, to split up the light from the spark, and thus to provide monochromatic illumination.
4. Sub-stage illuminators constructed of quartz to produce both dark-ground and transmitted light images.
5. Monochromatic objectives constructed of fused quartz, specially corrected for each wave-length it is proposed to use, together with suitable quartz oculars.
6. A solidly constructed microscope with an accurate and reliable fine-adjustment.
7. A camera suitably disposed so that it can be easily removed for purposes of observation or adjustment.
8. A solid base to which all the enumerated components can be rigidly fixed, together with a mercury-vapour lamp for preliminary observational purposes.

The source of light is a high-tension spark discharge between metallic electrodes, usually magnesium or cadmium. The necessary characteristics of the light are, that it should emit ultra-violet radiations in sufficient quantity, and that its spectrum should mainly consist of a few intense bright lines, or groups of lines. The lines must not be too close together, since it is a primary requirement that any individual line may be isolated by spectroscopic methods, and utilised as the source of light.

Few metals conform to the necessary conditions. Iron, for instance, is quite unsuitable, although it emits ultra-violet rays freely ; but the large number of lines in its spectrum, and their closeness together, make it impossible to utilise it. Cadmium and magnesium are the most suitable, their spectra consisting of a few intense well-separated lines. The length of spark is usually about 3 mm., but it must be what is often referred to as a ' fat ' type of spark of high intrinsic brilliancy. There is a definite limit to the amount of energy that can be used ; there appears to be a saturation point. The conditions are comparable to that of an arc, in which increase of current results in a greater volume of light owing to the increased size of the luminous crater, but does not materially alter the intrinsic brilliancy. The high-tension transforming system used is therefore one that gives a heavy discharge at a relatively low voltage. An oil-immersed condenser, which has been found superior to a Leyden jar, is connected in parallel with the spark, with the result that a rapidly alternating discharge takes place between the metal points.

An alternating current supply taken from the mains provides the most convenient source of power and eliminates the necessity for any form of interrupter in the primary circuit, but in our existing apparatus provision is now made for increasing the number of alternations as by this means exposures are substantially reduced. The metal electrodes are held in clamps supported on insulated pillars (Fig. 112) and the distance between them can be adjusted by means of the lateral screws seen at the base of the pillars. They are supported on a saddle carried on an optical bench which provides a means of altering their position in the direction of the optic axis of the illuminating system.

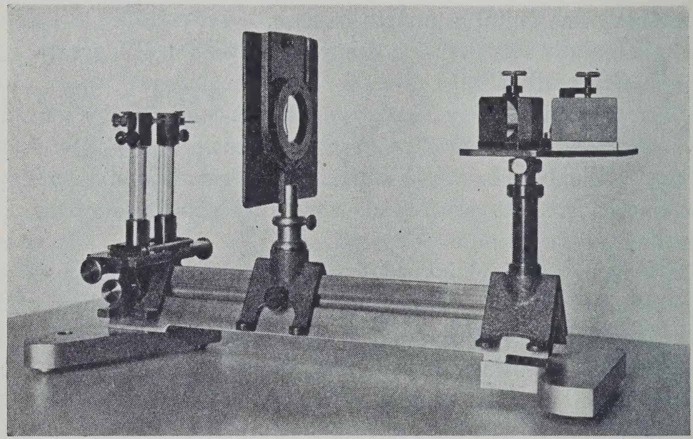


Fig. 112.

A quartz collecting-lens is placed as shown between the spark and the prisms, an image of the spark being projected in the plane of the back lens of the microscope condenser. The two prisms of quartz split up the light from the spark and provide monochromatic ultra-violet light of the required wave-length.

The optical bench carrying these units may be rotated about an axis passing through the last face of the second prism. Thus any available wave-length may be used for purposes of illumination by rotating the optical bench.

Sub-stage Illuminators.—The original apparatus designed by Professor Köhler embodied a quartz sub-stage condenser by means of which ultra-violet light of the required wave-length could be focussed in the plane of the object. The method was therefore similar to that described when using visible light, either the image of the iris-diaphragm on the collecting-lens, or the image of the face of the second prism could be focussed in the object plane, and centred in the usual way. Observations of these adjustments are made by means of the fluorescent searcher eye-piece, to which reference will be made. As a large part of the work done by the writers has been the identification of small living organisms other methods of illumination were devised to secure adequate visibility, which it was difficult to attain by means of transmitted light alone. The first illuminator designed by the writers provided a means of focussing the object in visible monochromatic light by dark-ground illumination and then changing over to transmitted ultra-violet light illumination. This condenser is a composite one and consists of a high-aperture dark-ground illuminator mounted concentrically with and encircling a quartz condenser. The former secures visibility in ordinary light and is used for purposes of search, location and primary focussing. The latter is available for use with any suitable ultra-violet radiation and enables a transmitted light image to be secured. Fig. 113 shows the appliance in section. Visible light enters as shown at LL, the centre of the beam being stopped out by a removable plug which allows only an annular beam to enter. This beam is twice reflected in the glass reflector A and reaches the object after passing through the front lens C of the central quartz condenser. Thus the object is seen illuminated on a dark-ground and can be focussed. When it is desired to photograph the object in ultra-violet light the central stop is removed and replaced by a tube carrying an annular stop which allows ultra-violet light K to enter the central quartz condenser only, E D C. The quartz object slide is shown at F. Thus the condenser is focussed on the object primarily in visible monochromatic light, and by simply changing the stops ultra-violet light is focussed on the object ready for photography. The foci of the two systems are similar, but exact par-focality is secured by actuating the knurled ring which adjusts the dark-ground illuminator only.

The use of the dark-ground method for ultra-violet work was first attempted with a dark-ground illuminator made for us by Messrs. R. & J. Beck, Ltd. This was the same in principle as the high-power illuminator already described in an earlier chapter, except that quartz and magnalium were substituted for glass and silver reflecting surfaces. The correction was excellent, but the loss of light, owing to the necessity of using a narrow annular illuminating beam, made the exposure so long as to be impracticable. Considerable experimental work was carried out, and finally a new form of condenser was designed and constructed.

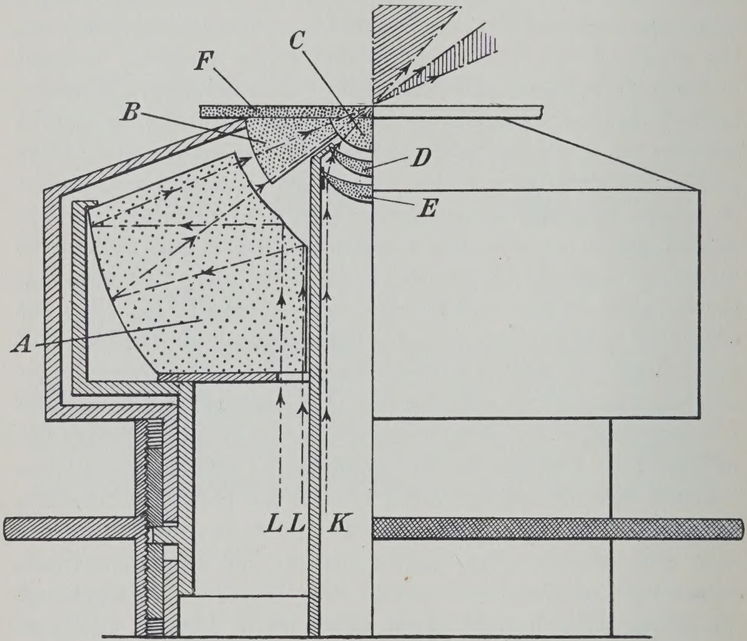


Fig. 113.

Fig. 114 shows in section the principle, the method of construction, and path of rays.

A beam of ultra-violet light is thrown on to the base of a quartz cone A, and is internally reflected on to an annular magnalium reflector B. The reflecting surface B, is of such curvature that the light is concentrated at D after passing through the quartz lens C. D is the position occupied by the object, and at that point practically the whole of the light passing through the quartz cone is focussed on the object. Thus there is no loss of light, such as occurs with any form of annular illuminator. This appliance has enabled us to obtain photo-micrographs of small living organisms in ultra-violet light by dark-ground illumination with exposures as short as 5 seconds. It also works well with visible light and is used for preliminary searching and focussing.

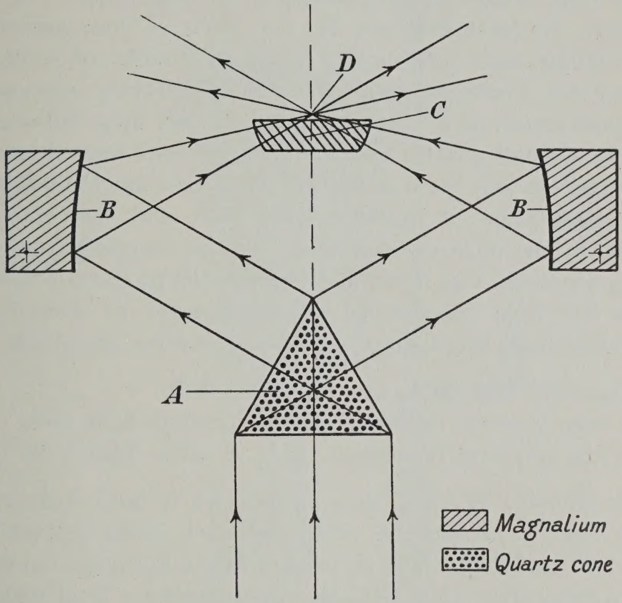


Fig. 114.

Quartz Objectives and Oculars.—The transparency of any optical glass is not sufficient for work with the ultra-violet portion of the spectrum. The only materials available therefore are quartz, either crystalline or fused, and fluorite. Fused quartz is generally rather less transparent than crystalline, but in carefully selected pieces which are free from impurities the difference is not important. Further improvements have recently been made in the production of fused quartz so that it is now of high quality in pieces large enough for objectives. Fluorite, which is highly transparent to ultra-violet light, was first used in the construction of microscope objectives by Spencer in America as long ago as 1860, while Boys in England, in or about 1885, suggested the suitability of fused quartz for this purpose. In 1900 Professor M. von Rohr of Jena succeeded in designing and constructing a quartz-fluorite objective of about 4 mm. equivalent focus, N.A. 0·30. This may be regarded as experimental, as it was supplanted in 1904 by a series made entirely of fused quartz. These objectives were termed ‘monochromats,’ as they were computed for a wave-length of 275 $\mu\mu$. The computation of objectives for other and shorter wave-lengths in the ultra-violet region was also successfully carried out by Professor von Rohr, and recently the production of such lenses has been successfully carried through by Zeiss. The equivalent focal lengths of the 275 $\mu\mu$ objectives are as follows :

6 mm. dry lens, N.A. in white light 0·35.
2·5 mm. glycerin immersion, N.A. in white light 0·85.
1·7 mm. glycerin immersion, N.A. in white light 1·25.

The effective N.A. of these lenses, when used with ultra-violet light is double the value indicated, being respectively 0·70, 1·70, and 2·50. The immersion fluid is glycerine and water with a refractive index 1·447/d. The immersion fluid must be sufficiently transparent to ultra-violet light, and in this respect such a mixture fulfils the purpose. In practice it is important to ensure that its refractive index remains constant as even a short exposure to atmospheric influence may cause appreciable alteration.

Five oculars for projecting the image on to the photographic plate have been computed, and these are made from crystalline quartz. Their magnifications are 5, 7, 10, 14 and 20 when used at a mechanical tube-length of 160 mm.

In the original design a photographic apparatus of the vertical type was used. The Zeiss vertical camera is the one usually recommended for this purpose, and is made in a suitable form with certain modifications from the ordinary type (Fig. 115).

For direct observation—that is, for finding the object and centring the image—a searcher fluorescent eye-piece A is employed. It is so arranged above the microscope that it can be used for observation and easily swung out of position when the camera is used; either appliance may therefore be used alternatively. In the searcher eye-piece is a small fluorescent screen, and a high-power magnifier is so arranged that the image formed on this screen may be observed directly. When the image has been focussed on the fluorescent screen, it will

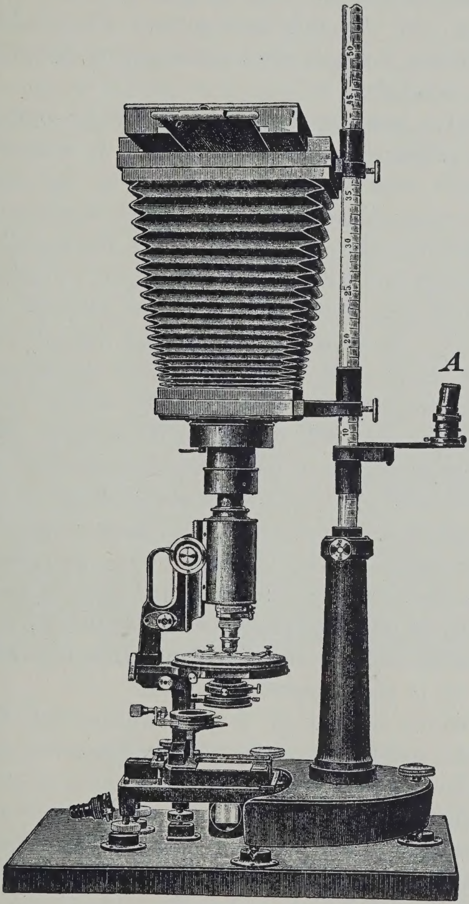


Fig. 115.

be in focus when the camera is placed in position and the photographic plate substituted. Variations in the length of the camera, not exceeding a few centimetres, will not have any considerable effect on the sharpness of the image. The disposition of the microscope camera and searcher eye-piece is shown in Fig. 115. In this arrangement ultra-violet light is reflected vertically through the microscope by the right-angled quartz prism beneath.

The original apparatus designed by Köhler embodied the essentials for ultra-violet microscopy, but it was difficult to use and results were uncertain. One of us did considerable experimental work with it and obtained some encouraging results. It became clear, however, that a microscope of more substantial design and with higher accuracy of adjustment must be produced if this method was to do all that might be expected of it. It is impossible in the limited space available here to describe the stages by which an apparatus was evolved; it must suffice to show its form as it is at this time. Further improvements will no doubt be devised, some are in course of development even now, but the apparatus to be described has sufficiently justified its existence in its use and application to the most difficult of present-day microscopical problems.

The microscope is of the horizontal type as it was realised that any vertical arrangement presented greater difficulties if rigidity was to be secured. Further, the setting up and adjustment of all the parts was easier and no reflecting quartz prism was needed as in the original apparatus.

Fig. 116 shows a view of the apparatus with the camera removed. It consists essentially of four parts:

(A) A massive iron bedplate.
(B) The microscope.
(C) The optical bench for illumination with visible light.
(D) The optical bench for ultra-violet illumination.

(A) *The main bedplate* is a heavily ribbed iron casting of great strength and rigidity. The microscope is placed at its narrow end fitting into three radiating slots, which accurately locate the three points of support of the microscope B and thus ensure its accurate alignment with the rest of the apparatus. The two optical benches (C and D) are fixed to the main bedplate. This bedplate is supported on three feet.

Figs. 117 and 118 show the microscope from each side. The coarse adjustment is actuated by the handle A and the fine-adjustment by the graduated milled-head B. Both the coarse and fine-adjustment controls operate one screw, the difference in the rate of movement is governed by a simple gear-box arrangement which can be thrown in or out of action by a lever C. The fine-adjustment milled-head is graduated into 100 divisions each of which represents a movement of the nose-piece carrying the objective of 0·0001 mm. (1/250,000 inch). This extremely slow movement is essential because of the small penetration of the quartz objectives, and to enable very minute objects to be focussed accurately. The sub-stage illuminators are carried on a rigid fitting on a dove-tail slide and this is controlled by a large milled-head D on which is a circle divided into 100 parts. Each division represents a movement of the sub-stage of 0·01 mm. and allows accurate setting of the sub-stage in the direction of the optic axis by reference to the graduation only. The sub-stage centring adjustments are actuated by the screws E, E, and the movements are controlled by dove-tail slides to ensure rigidity. All sub-stage illuminators are carried on interchangeable plates as described elsewhere in this book.

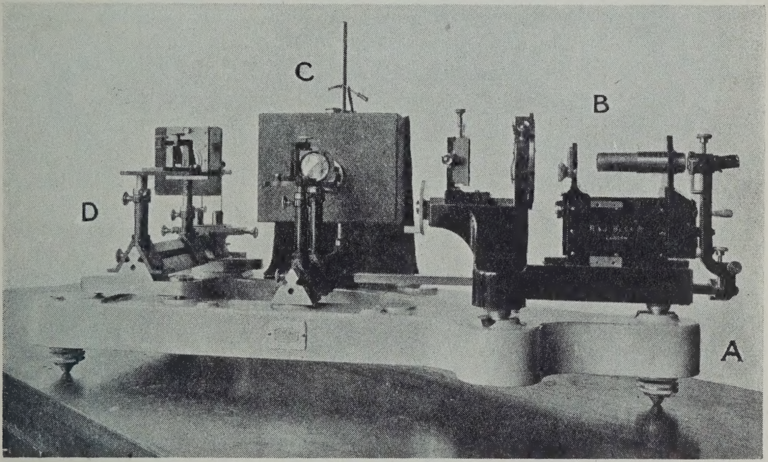


Fig. 116.

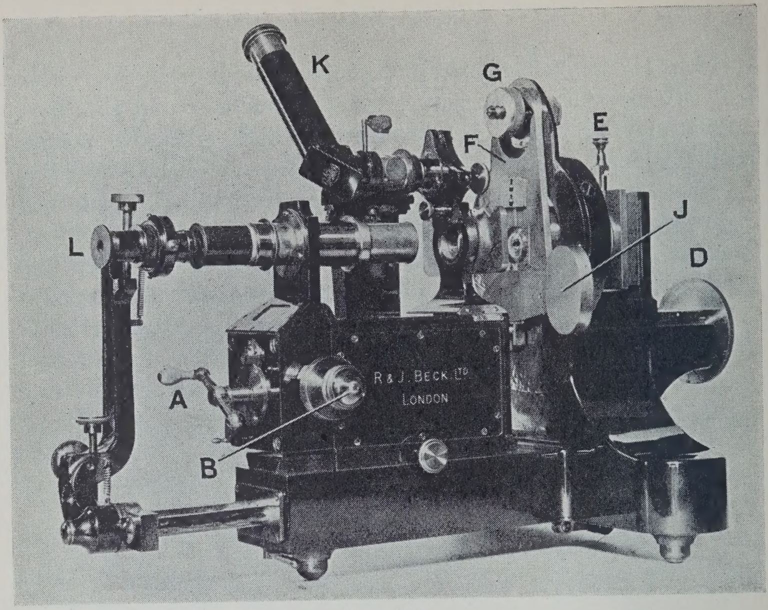

K
G
E
F
J
D
L
A
R & J. BECK LTD
LONDON
B

Fig. 117.

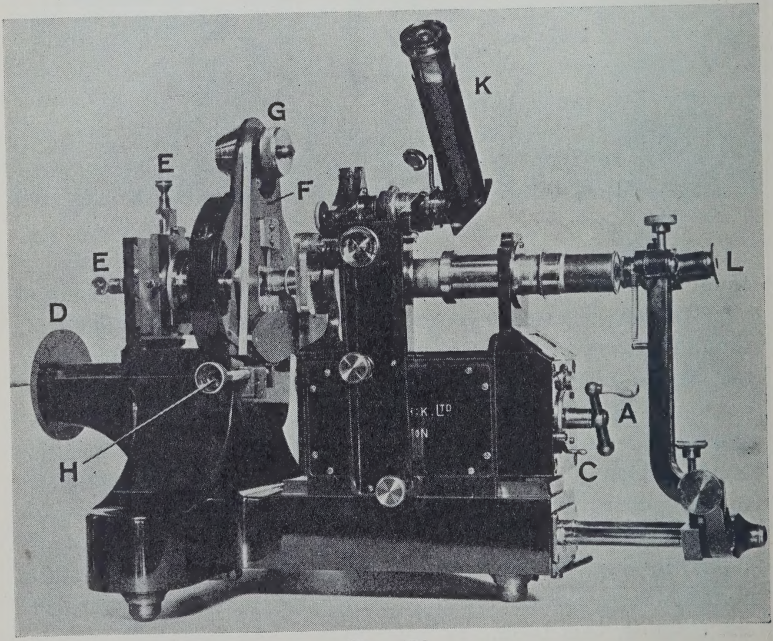
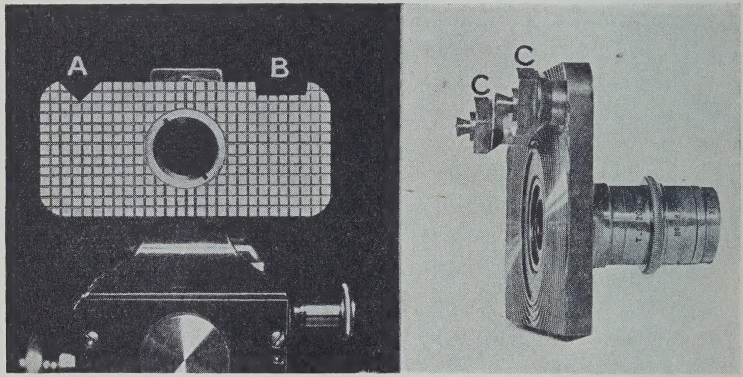

K
G
E
F
E
L
D
A
H
C

Fig. 118.

The mechanical stage is of novel design, and provides a means by which the stage with object on it can be transferred to another microscope if so desired without disturbing the centration or position of the object. It consists of a metal plate F suspended on a steel cone G which is carried on a screw and travels in or out when rotated. The plate hangs on the cone which as it is rotated causes the plate to rise or fall. This therefore provides the vertical movement for the stage and is very accurate. The hole in the plate where it rests on the cone is large enough to allow the plate to be easily lifted off. The horizontal motion of the stage is controlled by the micrometer screw H which engages on a projection near the bottom of the plate and causes it to have a swinging motion. The horizontal position of the plate is kept constant by gravity, the weight J on the side of the plate ensuring that it is kept up to the screw H. The quartz object-slide is supported on a ledge as shown and is held in position, in contact with the surface of the plate, by a small weight which moves vertically. This mechanical stage has been evolved as the result of much experimental work, and although simple in design it has proved to be entirely satisfactory in use.

The main feature in the apparatus is the method of securing interchangeability of the objectives as focussing in reference to the image in the fluorescent ocular is difficult and often impossible. The principle therefore is to focus the image in monochromatic visible light and then to change over to a quartz monochromat without the necessity for alteration of any adjustment. In practice the object is located and focussed and all optical adjustments made by means of a glass apochromatic objective using the green line emitted by the mercury-vapour lamp as the source of light. This lamp and condensing-lens are shown in Fig. 116 at C and the illuminating beam is deflected along the optic axis of the microscope by means of a glass right-angled reflecting prism not shown in the illustration. This prism is removed when a photograph is to be taken in ultra-violet light. The method of changing the objectives is simple in principle, but high accuracy must be secured. Each objective is carried on a flat plate, optically worked to a true surface, to the upper edge of which two steel cones are attached. Each of these cones rotates on an excentric axis and so provides a means of centring the objectives not only to the axis of the microscope but to each other. On the nose-piece of the microscope itself another flat plate is fixed on which the plates carrying the objective hang freely and therefore maintain their position by gravity. The cones act as hooks and the position of the objectives in relation to the axis of the microscope is determined by rotation of the cones. Once centration has been secured the cones are clamped and need no further adjustment. Fig. 119 *a* shows the plate on which the objective changer is supported on the microscope. This plate is screwed to the microscope nose-piece in the position usually occupied by the objective. Numerous grooves are cut on its optically worked face as shown. The **V** shape and oblong slots A and B are provided so that the cones on the objective holder drop into them and prevent movement except in a vertical direction. The position of the objective on the changer plate is shown in Fig. 119 *b*. This plate, on its face which comes in contact with the nose-piece plate, has a number of concentric grooves. The changer is placed in position with a sliding motion, so that any dust which might cause slight displacement and so prevent perfect contact, is swept into one or other of the grooves. The adjusting cones C are provided with a central locking device so that they may be fixed once centration has been secured. Par-focality is provided for by alteration of the thickness of the shoulder up to which the objective is screwed.



a Fig. 119. *b*

In Figs. 117 and 118, K is an interferometer which is attachable to the microscope and provides means by which it is possible to check the accuracy of the fine-adjustment motion, and if necessary to measure the thickness of any layer of material which it is desired to photograph. The fluorescent searcher ocular is shown at L and this can be thrown to one side when not needed. The camera is not shown in the illustration, but it is carried on a rigid arm, and is attached to the main base-plate A in a dove-tail slide machined for its reception. It is held by a quick-acting clamp and is easily attached or removed.

It may help to a better understanding of the procedure if a brief description is given of the sequence of events in taking a photo-micrograph. The glass reflecting prism is placed in position so that light from the mercury-vapour lamp is deflected into the microscope, a colour-screen being interposed to provide monochromatic green light. The sub-stage condenser is placed in position, centred and focussed in the usual way in relation to the object on the stage. A low-power objective is first used for preliminary adjustments, and then a 3-mm. glycerin immersion apochromatic objective is substituted. Both these objectives are carried on the interchangeable plates already described so that they are par-focal and centred to one another.

The preparation is searched for a suitable spot and the image is then focussed with the utmost accuracy. The glass objective is removed and a quartz monochromat is substituted. As it is impossible to make preparations of constant thickness or to ensure that any required element of structure is in actual contact with the under surface of the cover-glass, some small focussing latitude is allowed between the glass and quartz objectives. The difference, however, is usually within narrow limits and its direction is known. Some movement of the fine-adjustment is therefore made, but the amount of this movement can only be arrived at by experiment and experience. The glass reflecting prism is removed and the spark apparatus at D, Fig. 116, brought into use. The accurate adjustment of the spark and of the beam leaving the quartz prisms must be made in the first place so that the beam from the spark and from the mercury-vapour lamp have the same optical paths. The camera is attached, the fine-adjustment altered to the necessary position and the exposure made. The adjustment of the sub-stage and illuminating system for ultra-violet light is carried out by the aid of the fluorescent searcher eye-piece and this is its main function. We no longer use it for focussing or for observing the object.

Optically worked quartz slides and cover-glasses of definite thickness are used, the slides being made of crystalline and the cover-glasses of fused quartz.

In practice the preparation of the object presents no difficulty. The only point is that it must be mounted between quartz cover-glass and slide in some fluid which is transparent to ultra-violet light. Such fluids are not numerous; but distilled water, glycerin, or glycerin and water, Ringer's solution, normal saline, and castor oil are among the substances available.

It will be appreciated that in addition to the increased resolving-power, this method is in effect equivalent to another means of differential staining. All bodies to be photographed by this means must be unstained, and any detail of structure will be brought out as the result of differences in the absorptive-power of the tissue to ultra-violet light. The preparations must not be fixed, dried, or otherwise treated in the way that microscopical preparations are usually dealt with. The method is not a simple one, and at first the results may not prove satisfactory. It is essentially one that calls for the exercise of considerable patience and not a little skill, but the new microscope described so simplifies the technique that the production of photo-micrographs in ultra-violet light may now be attempted with confidence. Once the manipulation has been mastered it is possible to produce photographs of living tissues and organisms which cannot be secured in any other way.

It is impossible in the space available here to give more than an outline of this process, to deal with it adequately it would need a book to itself. The purpose is to enable those who are interested to get an idea of the apparatus and of the procedure in use.

CHAPTER XIII

SOME PROGRESSIVE EXAMPLES

HOWEVER full any general instructions may be, they cannot cover the whole of the varied conditions and circumstances that may arise. A series of progressive examples, therefore, in which various types of objects are selected and various methods of photographing them described, may prove more useful than a treatment of the subject on a broader basis.

All the photographs in Plates XII to XXIII are selected not merely as pictorial examples, but as types and varieties that may arise in practice. They do not claim to be the finest that can be produced of a particular subject, but in all cases they are fair examples of what may be expected from an object of average quality. It is a matter of common knowledge that many extremely fine examples of photo-micrographic work have been produced not only as the result of perfect technique, but also because the object itself happened to be of unusual quality and suitability. All those here delineated have been specimens of average quality such as any worker may expect to meet with in the usual course.

The description given is generally sufficient to indicate the procedure. Camera-length is the only factor that may require variation to obtain the same result. It has already been stated that lenses, even of the same nominal focal length, vary within moderate limits in magnifying power. This may be allowed for, and any necessary correction made by variation of the camera-length. Therefore, in some cases where an achromatic objective has been used in which the focal length does not accurately indicate its magnifying power, the camera-length has not been mentioned.

The objects are not placed in the absolute order of their difficulty. Some sacrifice has had to be made in this respect to enable them to be reproduced within the limits of the available space. The prints are from untouched negatives.

PLATE XII, FIG. 1.—**Transverse Section of Stem of Clematis.** × 20.

Double stained with carmine and iodine-green.

Objective, 35 mm., Zeiss apochromat, for direct projection without ocular.

Camera-extension, 2 ft. 9 in.

Illuminant, Pointolite lamp.

No sub-stage condenser.

Convergent beam from Nelson's achromatic bull's-eye, projected so that the image of the radiant was focussed approximately at the surface of the front lens of the objective.

Screen, saturated solution of picric acid.

Exposure, 5 seconds.

Plate, Imperial orthochromatic extra rapid.

Development by metol-hydrokinone.

PLATE XII, FIG. 2.—**Transverse Section of Stem of Beech.** × 20. Double stained, as above.

Objective, planar 42 mm., by James Swift & Son.

All other conditions as in Fig. 1.

Figs. 1 and 2 are photographed under exactly similar conditions to show the difference that exists between ordinary low-power objectives—even those of a high class, such as the apochromatic lenses—and the later types of low powers, such as planars and micro-summars. It will be seen on carefully looking at Fig. 1 that the definition falls off decidedly near the edge of the specimen although the central portion is perfect, whereas in Fig. 2 the definition at the edge is as good as at the centre. Both these photographs are but portions of the original negative, approximately only a quarter of the available field being shown in the reproduction. In photographing such preparations therefore it is necessary to use a lens which has a large field rather than one that possesses corrections of a high order confined to a smaller field.

PLATE XII

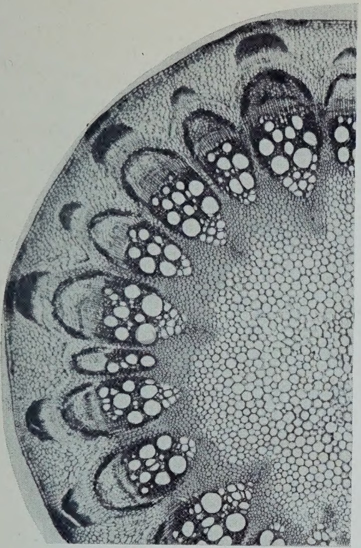


FIG. 1.

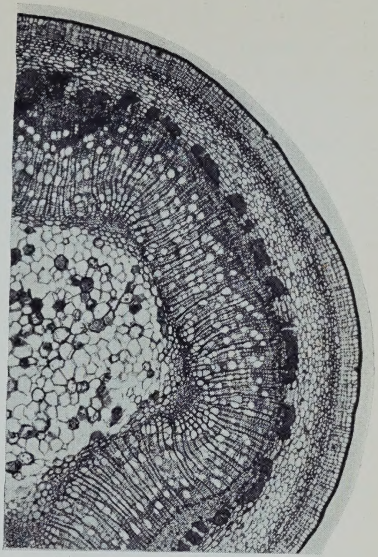


FIG. 2.

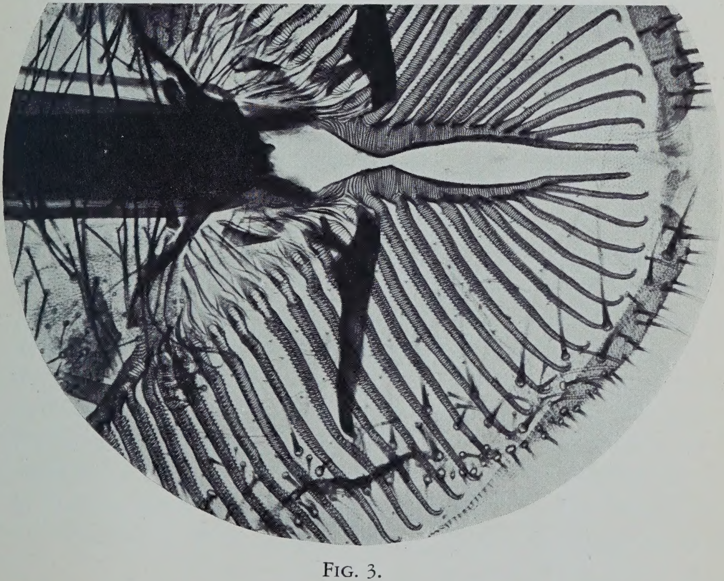


FIG. 3.

Plate XII, Fig. 3.—**Proboscis of Blow-fly.** × 100.

One-inch achromatic objective, N.A., 0·30, by James Swift & Son.

Mediar ocular, 3.

Sub-stage condenser, Swift achromatic with top combination removed. Working N.A., approximately 0·28.

Auxiliary condensers, Zeiss Nos. I and III.

Illuminant, mercury-vapour lamp.

Screen, Gifford F-line.

Camera-extension, 2 ft. 6 in.

Plate, Wellington orthochromatic process.

Exposure, 1½ minutes.

Development by pyro-soda, with half the normal quantity of pyro in solution, to reduce contrast in the dense part of the preparation.

This object is always regarded as a particularly good test with low-power objectives. The illumination should be effected with as large a cone of light as the objective will tolerate, and all corrections must be carefully made to ensure that the fine spirals and the outlying hairs on the tongue are brought out as perfectly as possible. The hairs themselves should, where they are in focus, die away to a sharp well-defined point. It is necessary to select a tongue that is mounted flat, and that is not opaque even in its densest parts; otherwise it is impossible to obtain detail in these parts without lessening that to be seen in the thinner parts. Its colour is usually so non-actinic that it may almost be regarded as an object in black-and-white, presenting no colour difficulty when orthochromatic plates are used.

Figs. 1 and 2 may be regarded as low-power examples. Neither of these would require the use of a sub-stage condenser; illumination could be by means of any suitable collecting-lens. The image of the illuminant should be focussed on the front lens of the objective and the only point necessary is to see that the field is evenly illuminated to the edge. To ensure this, correct centration of the light should be secured before the collecting-lens is introduced. The advantage of a well-corrected collector will be evident. Fig. 3 will require the use of a sub-stage condenser, and in this case its top lens should be removed if that is practicable. As the utmost resolution is required that the objective can give, the back lens of the condenser should be filled with light and the largest possible illuminating cone used. This should be determined by removing the ocular from the microscope and observing the back lens of the objective, which should appear evenly and fully illuminated. The exact plate used in photographing Figs. 1 and 2 may not now be obtainable but any similar plate may be used.

Plate XIII, Fig. 1.—**Colony on Gelatin Plate.** *Bacillus coli communis.* × 50.

Objective, 1-inch achromatic, by W. Watson & Sons.

No ocular.

Illuminant, electric arc, taking 5 amperes.

No sub-stage condenser.

Oblique illumination obtained by projecting a slightly convergent beam on to the mirror of the microscope, the latter being thrown well to one side.

Screen, Gifford F-line.

Camera-extension, 3 ft.

Plate, slow lantern, speed about 10. H. & D.

Exposure, 30 seconds.

Development by metol-hydrokinone.

In order to bring out the reticulated surface-structure suitable obliquity of the light is the essential point, and this can only be arrived at by experiment. The beam should first be projected at the smallest possible angle to the optical axis, and this angle gradually increased until the desired effect is obtained. The least possible obliquity of the beam should be used that will show up the structure, to avoid the introduction of diffraction effects that would otherwise interfere with the formation of a good image.

Plate XIII, Fig. 2.—**Transverse Section of Human Tongue.** × 50.

Stain, hæmatoxylin and eosin.

Objective, Zeiss 16-mm. apochromat.

Projection-ocular, 2.

Illumination, acetylene bicycle lamp.

Condenser, back combination of Swift achromatic condenser, working at its full N.A.

PLATE XIII

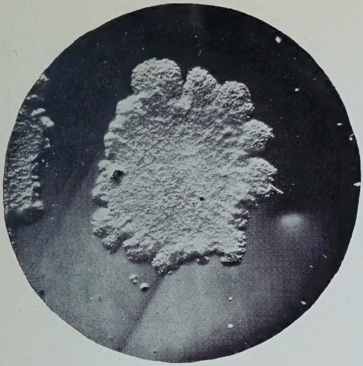


FIG. 1

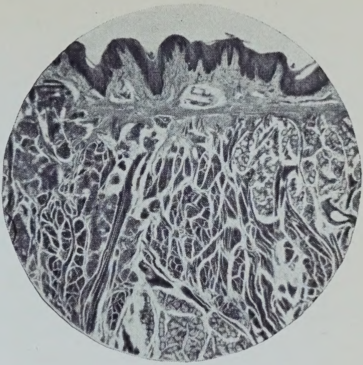


FIG. 2

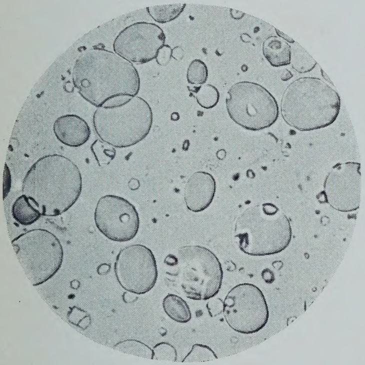


FIG. 3

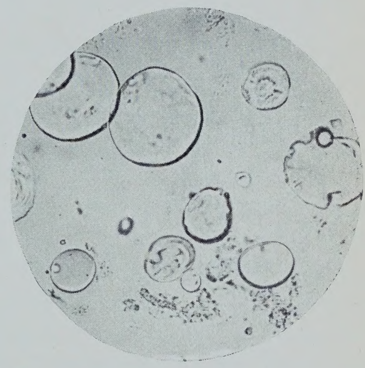


FIG. 4

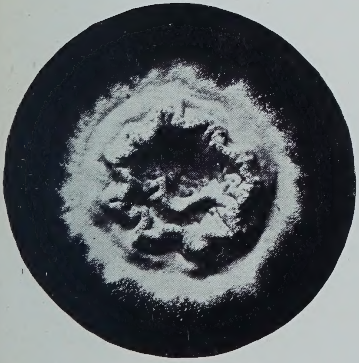


FIG. 5

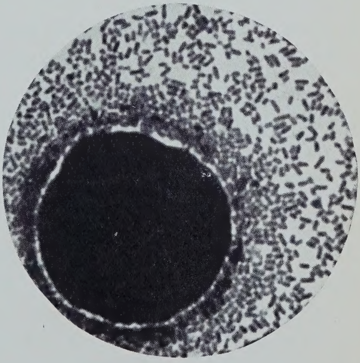


FIG. 6

Auxiliary condenser, Nelson achromatic.
Screen, saturated solution of picric acid.
Camera-extension, 20 in.
Plate, Imperial orthochromatic extra rapid.
Exposure, 15 seconds.
Development, metol-hydrokinone.

PLATE XIII, FIG. 3.—**Barley Starch.** × 350.
Objective, 8 mm. apochromat.
Projection-ocular, 4.
Illuminant, electric arc, 5 amperes.
Screen, green pot-glass.
Exposure, 5 seconds.
Plate, Wellington orthochromatic process.

PLATE XIII, FIG. 4.—**Rye Starch.** × 350.
Conditions as Fig. 3.

Figs. 3 and 4 are for purposes of comparison. The object is transparent but rather highly refractile. The illuminating cone cannot be large, as visibility must be secured; consequently the images show some evidence of diffraction round each granule. This must be reduced to a minimum so that the edge is as thin as possible consistent with a bright image. As the effective working aperture is therefore rather small centration should be perfect. In Fig. 3 this has been secured, but in Fig. 4 there has been slight decentration of the sub-stage condenser with the result that the image has thicker lines on one side, although there is no evident unequal illumination. Such objects are valuable for educational purposes, as the result of any error is seen in the photograph.

PLATE XIII, FIG. 5.—**Surface Colony of Ringworm Organism.** × 2.

Objective, 6¼-inch Zeiss double-protar anastigmat, on front panel of vertical, without microscope.

Illuminant, lime-light. A parallel beam projected on to surface of culture plate by ordinary bull's-eye, at an angle of 45° to the optical axis.

Screen, green pot-glass.
Plate, orthochromatic, medium rapidity.

Exposure, 10 seconds.
Development, pyro-soda.

Plate XIII, Fig. 6.—**Edge of Colony of** *B. suipestifer.* × 1250.

The colony was grown on a microscope slide on which a thin layer of culture medium was spread. After suitable incubation, the colony was stained *in situ* with fuchsin.

Objective, 3-mm. Zeiss apochromatic, N.A. 1·40.
Ocular, Zeiss projection, No. 4.
Illuminant, D.C. Automatic Arc-Lamp, 6 amperes.
Condenser, Zeiss achromatic with Zeiss 1 C. collecting-lens.
Colour-screen, Wratten B.
Plate, Ilford ortho-process, H. & D. 30.
Camera-extension, 100 cm.
Exposure, 1 second.
Development, pyro-soda.

The organisms are stained red and therefore a green colour-screen is used. This illustrates the importance of preparing the object for photography. Unless the organisms were grown on a flat surface and in one plane, it would be impossible to obtain such a result at this magnification.

Plate XIV, Fig. 1.—**Influenza Bacillus (Pfeiffer) in Sputum.** × 1000.

Preparation stained fuchsin.
Objective, $\frac{1}{12}$-inch achromatic by W. Watson & Sons.
Projection-ocular, 4.
Illuminant, Pointolite lamp.
Screen, Zetnow solution.
Plate, Wellington orthochromatic process, H. & D., 100.
Exposure, 30 seconds.
Development by pyro-soda, factorial method.

This organism is among the smallest recognised bacteria. The preparation is rather thick, and the advantage of selecting a spot where the bacteria are in one plane is well shown.

Plate XIV, Fig. 2.—**Koch's Spirillum of Cholera.** × 1500.

Cover-glass preparation of a pure culture.
Stain, fuchsin.

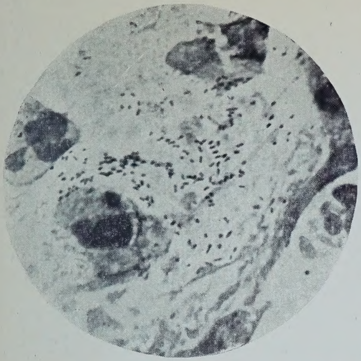


FIG. 1

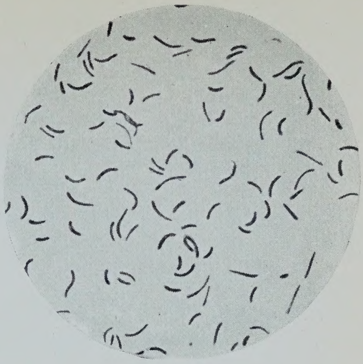


FIG. 2

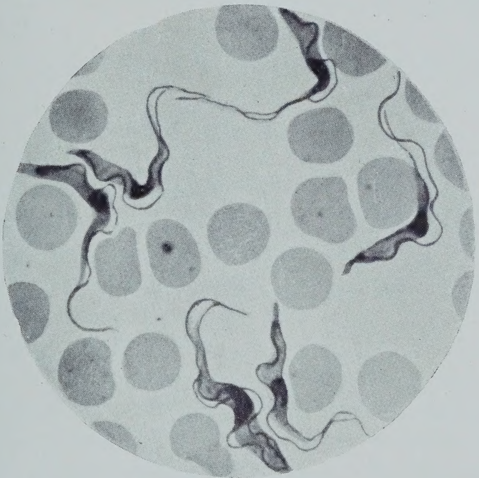


FIG. 3

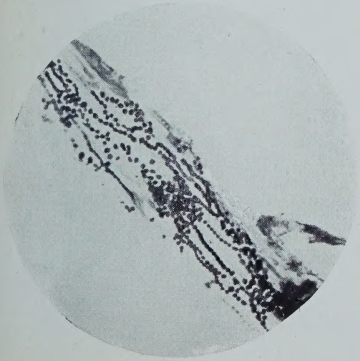


FIG. 4

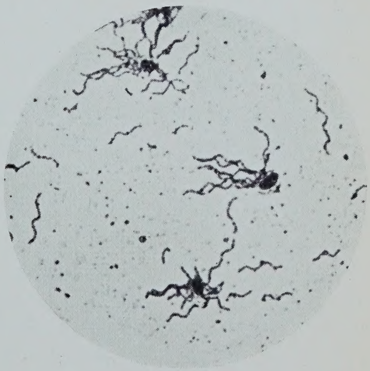


FIG. 5

Objective, 3-mm. apochromat, by Carl Zeiss, N.A. 1·40.
Projection-ocular, 2. Long camera-extension.
Illuminant, arc lamp, taking 4 amperes.
Condenser, Leitz oil-immersion, N.A. 1·40.
Auxiliary collecting-lens, Nelson achromatic bull's-eye.
Screen, iodine-green saturated solution.
Plate, Wellington orthochromatic process, H. & D., 100.
Exposure, 5 seconds.
Development, pyro-soda.

This is a typical example of a bacteriological preparation. Such are commonly met with in laboratory practice.

PLATE XIV, FIG. 3.—**Trypanosome.** *Trypanosoma gambiense.* × 1750.

Stained by Romanowsky-Leishman method. (Methylene-blue-eosin.)

$\frac{1}{12}$-inch objective, by C. Baker.
Projection-ocular, 4.
Abbe chromatic condenser.
Plano-convex auxiliary lens giving a parallel beam.
Illuminant, mercury-vapour lamp.
Green Wratten monochromatic screen.
Plate, Barnet orthochromatic.
Exposure, 5 minutes.
Development with pyro-soda.

Such preparations are often rather faintly stained although they show fine detail. Illumination must therefore be arranged to give the utmost attainable contrast. In this case the use of a mercury-vapour lamp, together with a suitable green screen giving truly monochromatic light, enables a result to be obtained that could hardly be secured by any other method.

PLATE XIV, FIG. 4.—**Ringworm on Human Hair.** × 450.

Preparation stained Gram's method, i.e. organisms deep violet, hair pale yellow.

Objective, $\frac{1}{6}$-inch achromatic by J. Swift & Son.
Ocular, No. 2 mediar.
Illuminant, lime-light.
No auxiliary condenser.
Screen, malachite green solution in glycerine.

Plate, Wellington orthochromatic process, H. & D., 100.

Exposure, 25 seconds.

Development by metol-hydrokinone.

The difficulty in this preparation is that there are many layers of organisms encasing the hair. An ordinary Abbe condenser was used, the iris-diaphragm contracted to increase penetration, but not far enough to cause diffraction effects round the images of the organisms. The particular point at which this happens must be looked for carefully.

PLATE XIV, FIG. 5.—**Cover-glass Preparation showing Flagellæ.** *Bacillus typhosus.* × 1250.

Staining by Van Ermengem's method.

Objective, 3-mm. apochromat, N.A. 1·40, by Carl Zeiss.

Projection-ocular, 4.

Illuminant, electric arc, taking 6 amperes.

Condenser, Swift achromatic.

Auxiliary collecting-lens, Zeiss No. I.

Screen, Gifford F-line.

Plate, Wellington ortho-process.

Exposure, 2 seconds.

Development, pyro-soda.

PLATE XV, FIG. 1.—**Spirochæte.** *Spironema Pallidum.* × 1000.

Stained Fontana's method. The organism appears brownish-black on a yellowish ground. The method is rather a deposition than a true staining process.

Objective, 3-mm. apochromat by Zeiss, N.A. 1·40.

Projection-ocular, 4.

Illuminant, arc lamp, taking 4 amperes.

Condenser, Zeiss achromatic.

Auxiliary collecting-lens, Zeiss aplanatic, 1 C.

No screen.

Plate, Wellington process.

Exposure, 2 seconds.

Development, metol-hydrokinone.

The relatively long exposure is due to the density of the stained organisms and the yellowness of the deposit over the whole preparation.

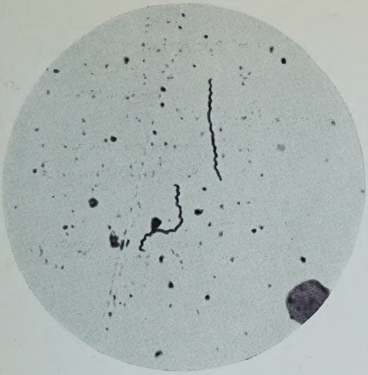


FIG. 1

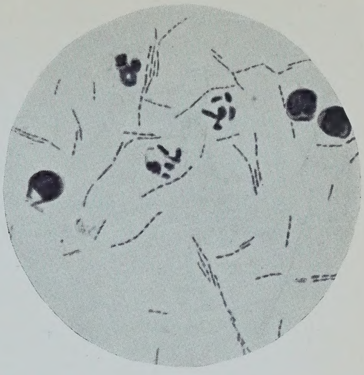


FIG. 2

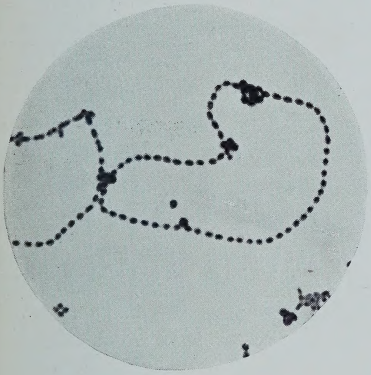


FIG. 3

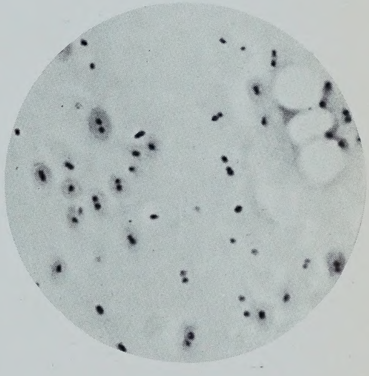


FIG. 4

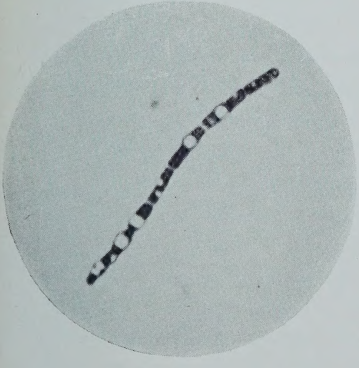


FIG. 5

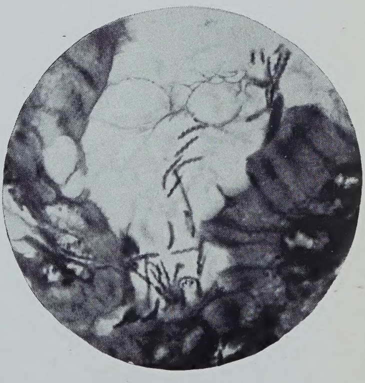


FIG. 6

Plate XIV, Fig. 2.—*Bacillus anthracis.* **Cover-glass Preparation of Blood of Inoculated Guinea-pig.** × 750.

Stain, Gram and eosin.
Objective, $\frac{1}{15}$-inch oil-immersion, by C. Reichert.
Ocular, none.
Illuminant, mercury-vapour lamp.
Condenser, Swift achromatic.
No auxiliary collecting-lens.
Screen, acid-green and tartrazine.
Plate, Barnet orthochromatic.
Exposure, 45 seconds.
Development, pyro-soda.

Plate XV, Fig. 3.—*Streptococcus pyogenes.* × 1250.

Cover-glass preparation from pure culture.
Stain, methyl-violet.
Objective, $\frac{1}{12}$-inch achromatic, by C. Baker.
Ocular, none.
Illuminant, electric arc, taking 4 amperes.
Condenser, Zeiss-Abbe chromatic.
Auxiliary collecting-lens, Nelson aplanatic bull's-eye.
Screen, aqueous solution of naphthol-green.
Plate, Wellington process, orthochromatic.
Exposure, 6 seconds.
Development, pyro-soda.

Plate XV, Fig. 4.—*Diplococcus pneumoniæ* **in Peritoneal Fluid of Mouse.** × 1200.

Cocci stained deep blue, capsule appears paler blue.

The whole ground is covered with the partially stained fluid which gives a grey background in the photograph.

Objective, Beck 2-mm. apochromatic, N.A. 1·3.
Ocular, projection 4.
Illuminant, Pointolite.
Condenser, Beck achromatic.
Screen, Wratten G.
Plate, Wellington ortho-process.
Exposure, 15 seconds.
Development, pyro-soda.

The difficulty in photographing a preparation such as this is to get sufficient difference in gradation between the organisms, the capsules, and the ground substance. This can only be secured by careful selection of a suitable colour-screen.

Plate XV, Fig. 5.—*Bacillus anthracis.* **Cover-glass Preparation from Pure Culture showing Spores.** This is an enlargement from a negative taken at × 1280, and enlarged twice to × 2560.

Plate XV, Fig. 6.—*Spirochœtes* **in Stomach of Bitch.** × 1000.

Conditions same as Fig. 1. The section was rather thick and the chief difficulty was to find a level spot. Such a preparation should be photographed at highest possible N.A., so that best resolution is obtained only in one plane, and the organisms out of focus are not apparent.

Plate XVI, Fig. 1. **Spine of an Echinus.** × 40.

Unstained preparation.

Objective, 1-inch achromatic, by R. & J. Beck, Ltd.

No ocular.

Camera-extension, 3 ft. 2 in.

Small tube of black paper slipped into body-tube of microscope to prevent reflection from the internal surfaces.

Dark-ground illumination obtained by putting a stop of suitable size at the back of the achromatic condenser, the top lens of which had been removed.

Illumination by Welsbach incandescent gaslight, the source of light being placed about eight inches from the sub-stage condenser.

No colour-screen.

Ilford ordinary landscape plate.

Exposure, 2 minutes.

Development by pyro-soda, the utmost contrast being secured by using an excess of pyro, and about 10 per cent. less than the normal quantity of carbonate of soda.

An example of low-power dark-ground illumination.

Plate XVI, Fig. 2.—*Bacillus megatherium.* × 1150.

Dark-ground illumination by means of Swift concentric illuminator.

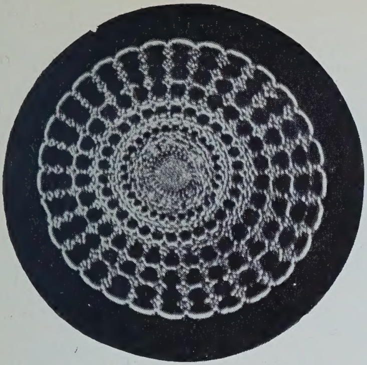


FIG. 1

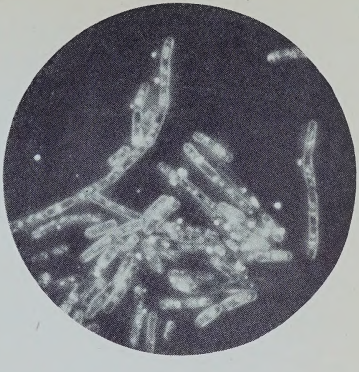


FIG. 2

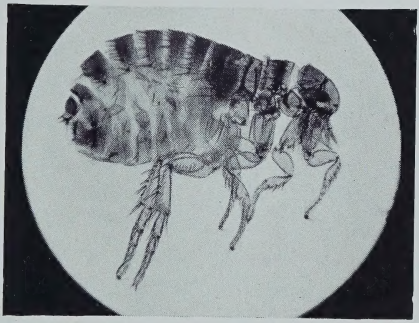


FIG. 3

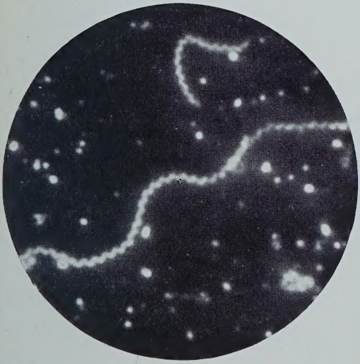


FIG. 4

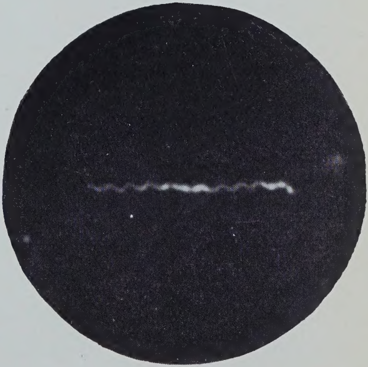


FIG. 5

Objective, Leitz, 1/7a.

Ocular, No. 4 projection. Photographed with reflex camera on Van Heurck apparatus (see page 134).

Illuminant, 5-ampere automatic arc lamp, by Zeiss, with aplanatic collecting-lens throwing a parallel beam on to microscope mirror.

Colour-screen, filter blue-green solution in cell.

Plate, Imperial special rapid.

Development by pyro-soda.

Plate XVI, Fig. 3. **Dog Flea.** × 16. Taken by Phoku Camera (Zeiss).

Objective, a.a.

Screen, Wratten B.

An example of a small negative suitable for subsequent enlargement.

Plate XVI, Fig. 4.—**Spirochæte:** *Leptospira Icterohæmorrhagiæ.* × 2500.

Dark-ground illumination by means of concentric illuminator by R. & J. Beck, Ltd. N.A. 1·25 to 1·40.

Objective, Beck 2-mm. apochromat, N.A. 1·20.

This spiral can only be resolved perfectly with this illuminator and objective, as any less N.A. is insufficient. Centration must be very accurately secured, as the illuminating beam is of such wide angle. The organism shows as a row of dots unless adjustment of all parts is perfect. High magnification is necessary, otherwise the true structure is obliterated in the reproduction process.

Plate XVI, Fig. 5.—**Spirochæte:** *Spironema Pallidum.* × 2500.

Dark-ground illumination by Leitz concentric illuminator.

Objective, 8-mm. apochromat.

Ocular, × 12, compensating. Van Heurck camera with reflex camera attached (see page 134).

Colour-screen, ammonio-copper sulphate.

The illustration is an enlargement from the original negative and shows that with certain objects a good result is secured more easily with a medium-power objective and subsequent enlargement, particularly with dark-ground images. Accurate adjustment for tube-length is essential and depends not only on cover-glass thickness but also on the thickness of the layer of material. It can only be carried out by observation of the image. It is important to use cover-glasses that are free from defects and that have been carefully cleaned. This applies to all dark-ground work, particularly when using a dry lens.

Figs. 2, 4, and 5 are photographs of living objects suspended in a medium of a watery nature. Some steps are therefore necessary to secure immobility. In all these cases the preparation has been allowed to rest for an hour or more before photographing.

PLATE XVII, FIG. 1.—**Group of Foraminifera.** × 25. Opaque object.

Objective, 35-mm. apochromat for direct projection, by Carl Zeiss.

No ocular.

Camera-extension, 2 ft. 9 in.

Illuminant, 4-ampere electric arc lamp.

The object is mounted as an opaque one, and illumination therefore has to be effected by obliquely incident light. The illuminant was arranged in a position to one side of and about fifteen inches away from the preparation, so that a beam of light at about an angle of 45° to the normal was projected on to it. An ordinary bull's-eye condenser was interposed, so that an enlarged image of the illuminant was focussed on to the preparation.

Exposure, 1½ minutes.

Slow lantern-plate.

Development with pyro-ammonia.

Such low-power opaque objects present no particular difficulty, except that the preparation should be mounted so that all the units as nearly as possible lie in the same plane. Where the object is one of considerable depth, it is better to have recourse to a lens of the planar type which has an iris-diaphragm. This allows the aperture to be reduced, with resulting increase of penetration. Alternatively, a Davis diaphragm which can be closed down may be placed at the back of the micro-objective, care being taken that the quality of the image is not thereby interfered with. In any case, for reasons already

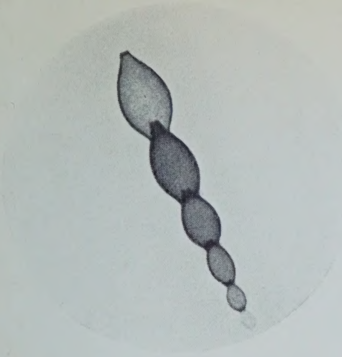


FIG. 2

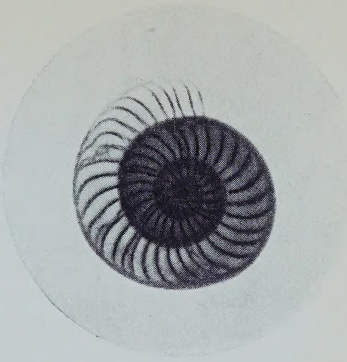


FIG. 3

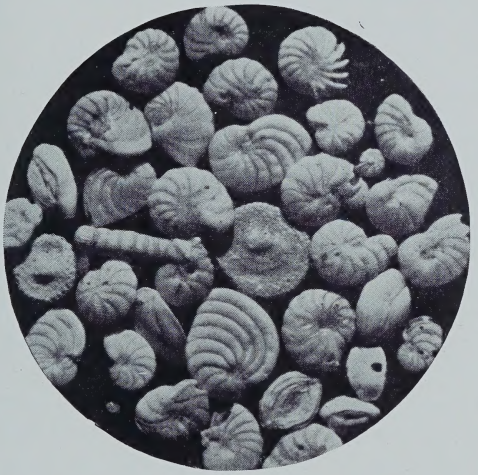


FIG. 1

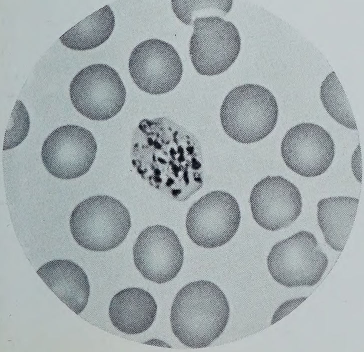


FIG. 4

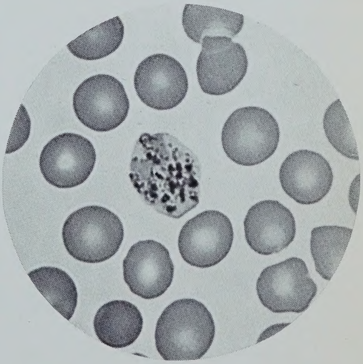


FIG. 5

stated, the lens should be of the lowest power possible. Another method, when the object has considerable depth, is to photograph at the lowest practicable magnification and subsequently to enlarge by ordinary photographic means.

It may be necessary to vary the angle of incidence of the light according to the depth of the surface-markings on each unit of the preparation. The nearer the incident-beam approaches to the vertical, the less the contrast that will be obtained. It is, however, advisable to keep the incident beam at as small an angle to the vertical as practicable; otherwise heavy shadow effects are caused on one side which interfere seriously with correct delineation of the object. These heavy shadows may be reduced by placing a reflecting surface, such as white paper, near to the object on the side opposite to the illuminant.

PLATE XVII, FIGS. 2 and 3.—**Varieties of Foraminifera,** as shown in Fig. 1.

The photographs were obtained by means of X-rays by the method described on page 290.

PLATE XVII, FIGS. 4 and 5.—**Malaria.** Blood film. × 1000. Stained Leishman's method.

Objective, 2-mm. apochromat by W. Watson & Sons, Ltd.

Ocular, projection, 4.

Screen, cupric nitrate and chromic acid.

The purpose of these two photographs is to show the effect of reducing unduly the aperture of the sub-stage condenser. Fig. 4 is taken at full aperture with the result that the contained granules are perfectly defined and free from diffraction effects. Fig. 5 is a photograph of the same field with the condenser aperture reduced. It will be seen that the granules and the red blood corpuscles are less well defined. The granules also appear larger than they should, another effect that is often seen with such objects under such conditions. The red blood cells frequently show an ill-defined edge and encircling ring which also is an optical effect due to too small an aperture.

PLATE XVIII, FIGS. 1, 2 and 3.—**Root Tip of Onion showing Mitosis.** × 450.

Objective, 8-mm. Zeiss apochromat.

Screen, naphthol green, solution in water.

These three photographs are to show that there need be little difference in the result whatever the light-source used. Fig. 1 was taken with D.C. arc, Fig. 2 with A.C. arc and Fig. 3 with gas-filled lamp and glass rod as described on page 107. There is no detectable difference between the first two, but the exposure with A.C. was nearly double that with D.C. In both cases an aplanatic collecting-lens was used in front of the arc in the usual way. With Fig. 3 the end of the glass rod was brought close to the sub-stage condenser without any collecting-lens. The exposure was 10 minutes. The result is practically as good as the earlier figures and might have been equal to them had a colour-screen of less density been used, comparable to the lower light intensity. The only difference seen is due to decreased penetrating power of the light used, there is no difference in definition.

PLATE XVIII, FIGS. 4 and 5.—**Diatom :** *Amphipleura pellucida.* × 1500.

Preparation mounted in realgar.

Objective, 2-mm. apochromat, N.A. 1·40 (corrected for 250-mm. tube-length), by Carl Zeiss.

Ocular, No. 4 projection.

Illuminant, electric arc, 10 amperes.

Condenser, Leitz oil-immersion, N.A. 1·40.

Auxiliary collecting-lens, Zeiss No. I.

Screen, aqueous solution of methylene-blue with signal-green glass to cut out the red otherwise transmitted.

Plate, Imperial orthochromatic.

Exposure, 4 minutes.

Development with metol-hydrokinone.

To bring out the lined structure illumination must be oblique, that is in the direction of the length of the object. This may be achieved by putting a slotted stop at the back of the sub-stage condenser in the manner already described, or by partially closing the iris-diaphragm and racking it across the field (as may be done in Continental forms of stands) so that only a small portion of the total aperture of the condenser, and that at one side only, allows light to pass. The exact amount of decentration of the sub-stage iris must be determined by experi-

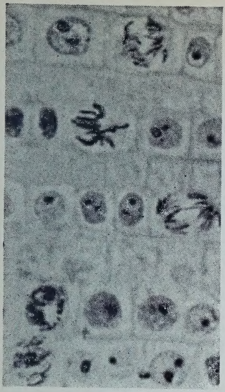


FIG. 1

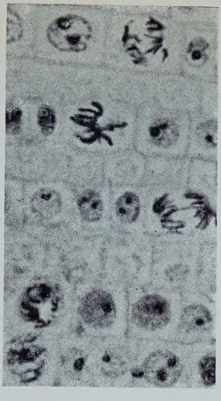


FIG. 2

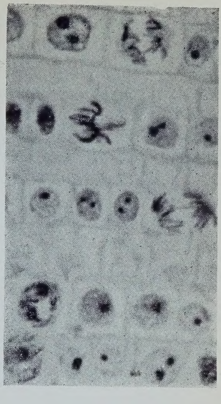


FIG. 3

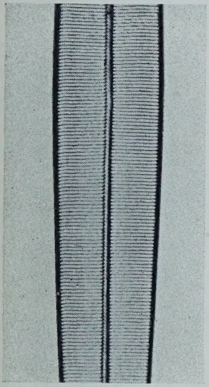


FIG. 4

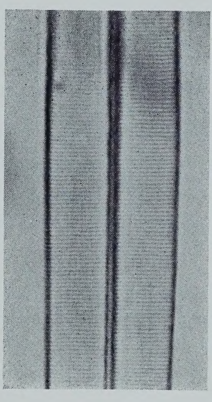


FIG. 5

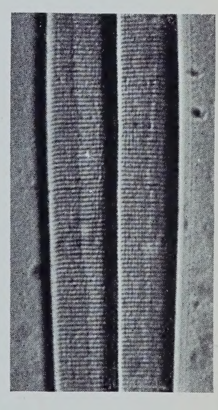


FIG. 6

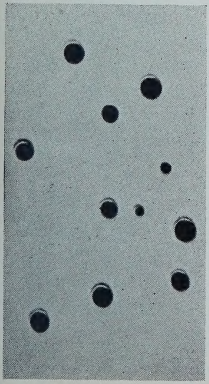


FIG. 7

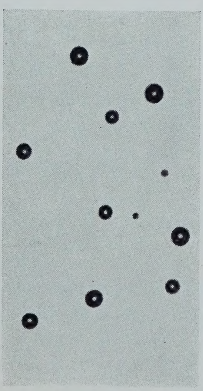


FIG. 8

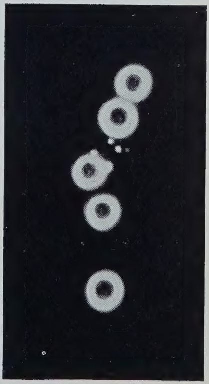


FIG. 9

ment, and depends to some extent on the character of the particular specimen. The largest opening and the least possible amount of decentration should be used, but the lines must be shown clear and bright. The black lines should not be more than one-fourth of the width of the intervening clear spaces.

PLATE XVIII, FIG. 6.—**Diatom:** *Amphipleura pellucida.* × 1500.

Exactly the same conditions of illumination obtained as in Figs. 4 and 5, except that the sub-stage iris-diaphragm is rotated so that instead of the illumination being in the direction of the length of the diatom it is approximately at an angle of 45° to it. Very few will show the dotted image at all, and it is only in exceptional specimens that it is possible to bring it out. The one chosen should be as large as possible, and it will be found that if the specimen is such that lines can be shown by illuminating at right angles to the length, the dots may be seen by suitably varying the incidence of the light. Perhaps the best method is to set up the object on a microscope with a rotating stage, arrange the illumination so that either transverse or longitudinal lines are shown, and then to rotate the stage carefully until the dotted image is seen with more or less distinctness.

The photography of diatoms is often regarded as difficult, but in fact it is not more so than most other branches of microscopic work. The provision of a suitable preparation is essential and in general it is necessary for the object to be mounted in a medium of high refractive index. Axial illumination is to be preferred and a small illuminating cone is permissible in nearly all cases. This is due to the fact that the diatom consists of nearly pure silica, which has a higher refractive index than that of most other microscopic objects. The effect is that, although the illuminating cone of light may be small, the actual angle of light entering the objective is large and may approximate to its full working aperture.

PLATE XVIII, FIGS. 7 and 8.—**Mercury droplets,** deposited by evaporating a small quantity of mercury in a watch-glass, and allowing the mercury vapour to condense on a clean glass slide. By varying the distance between the heated mercury and the surface of the slide smaller or larger droplets may be obtained of nearly uniform size. The preparation is mounted in Canada balsam beneath a cover-glass. × 1000.

Objective, $\frac{1}{12}$-inch, by James Swift & Son.

The two photographs are to show the effect of slight decentration of the illuminating system. This object is particularly sensitive to small errors of this sort and is therefore perhaps the best to use for test purposes. Fig. 7 shows the decentration effect while Fig. 8 is of the same field under correct conditions. The white central spot is an indication of perfect centration.

Plate XVIII, Fig. 9.—**Mercury droplets,** as above. × 2000.

Dark-ground illumination in which any errors of centration will be even more evident than with transmitted light. This object is of great value for experimental purposes and for testing the accuracy of the setting up of photo-micrographic apparatus. Most of the mercury globules are the same size, but a group of very small ones will be seen near the centre which are at the limit of resolution for the objective used.

Plate XIX, Fig. 1.—**Steel casting.** × 5. Showing cavities and decarburised edge.

Etched in 5 per cent. Nitric acid in Alcohol.
Objective, 3-inch Aldis Micro-anastigmat.
Green screen.
Ilford screened chromatic plate.

Plate XIX, Fig. 2.—**Nickel-Iron-Aluminium-bronze.** × 100.

Etched Ferric Chloride.
Objective, Watson $\frac{1}{2}$-inch parachromatic.
Screen Wratten K3.
Ilford screened chromatic plate.

Plate XIX, Fig. 3.—**Carbon steel.** × 250.

Etched 1 per cent. Nitric acid in Alcohol.
Objective, Watson 8-mm. apochromat.
Deep blue screen.
Ilford Press ortho plate.

ERRATUM

Plates XIX & XX should be numbered XX & XIX

To face p. 328. *Barnard and Welch, Practical Photo-Micrography*

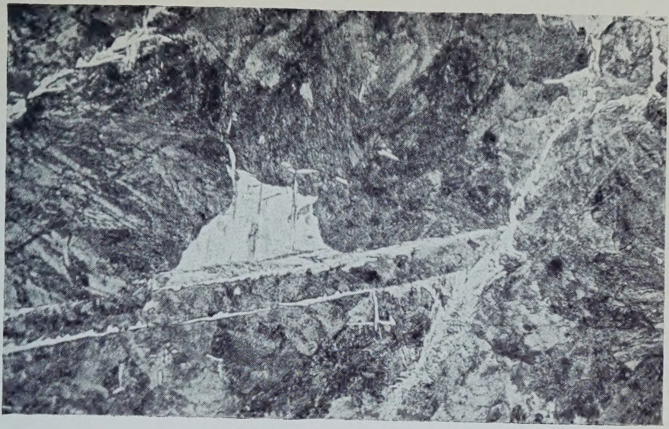


FIG. 1

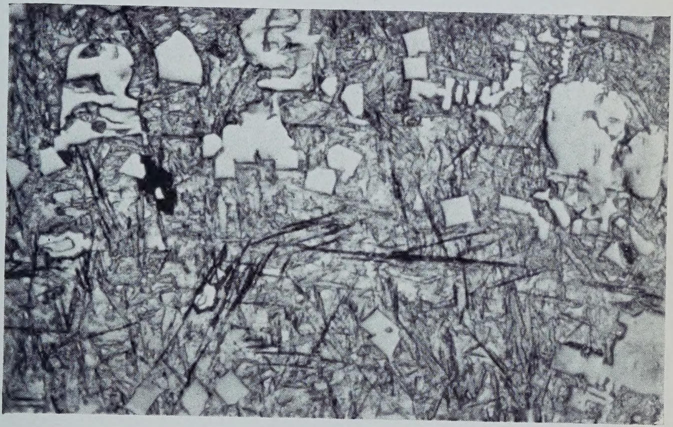


FIG. 2

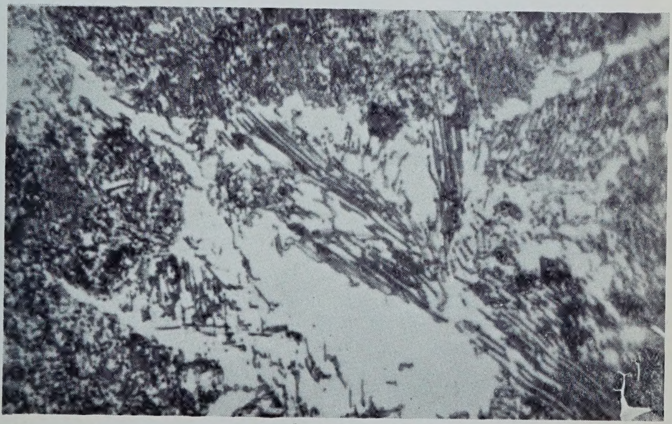


FIG. 3

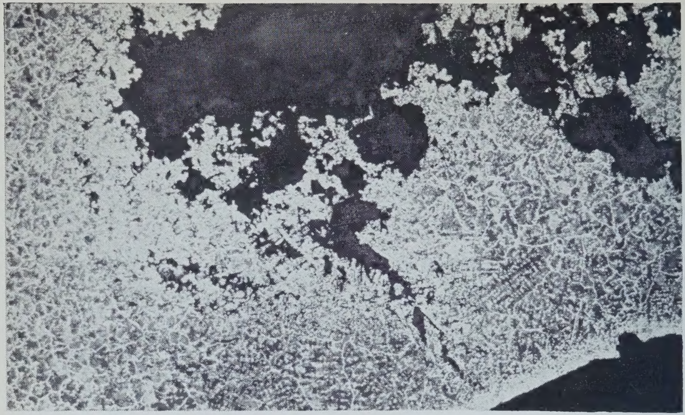


FIG. 1

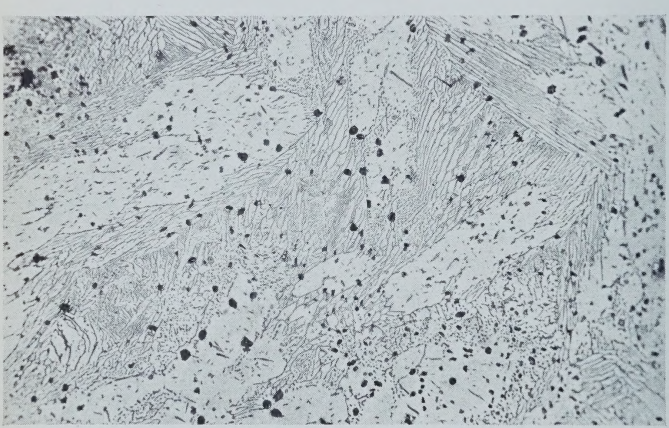


FIG. 2

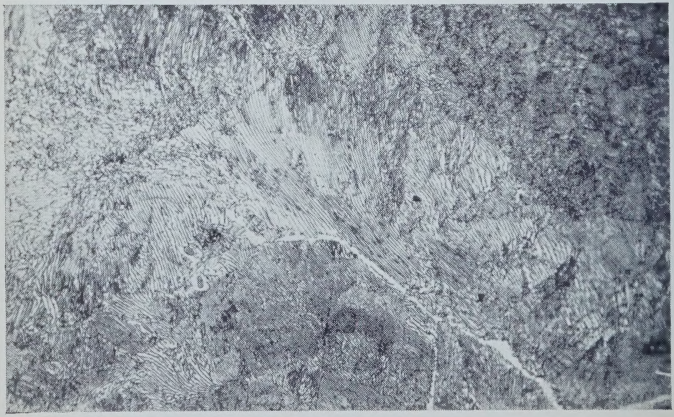


FIG. 3

Plate XX, Fig. 1.—**Structure in head of a steel rivet.** × 500. Showing grain of Martensite surrounded by Pearlite.
Etched 1 per cent. Nitric acid in Alcohol.
Objective, Beck 4-mm. apochromat.
Deep blue screen.
Ilford Press ortho plate.

Plate XX, Fig. 2.—**Iron Tantalum Alloy.** × 1000.
Etched 5 per cent. Nitric acid in Alcohol.
Objective, Leitz 3-mm. apochromat.
Deep blue screen.
Ilford Press ortho plate.

Plate XX, Fig. 3.—**Medium Carbon Steel.** × 2500.
Structure of Sorbitic Pearlite and Ferrite.
Etched 1 per cent. Nitric acid in Alcohol.
Objective, Beck 2-mm. monochromat 1·60 N.A.
Deep blue screen.
Ilford Press ortho plate.

We are indebted to Mr. H. Wrighton, B.Met., of Woolwich Arsenal, for the photo-micrographs on Plates XVIII and XIX, together with the description.

Plate XXI, Fig. 1.—**Cross-section through Finger-nail and surrounding Tissue of an Infant.** × 30.
Stained deep red and blue.
Zeiss a.a. objective.
No ocular.
Green screen, Wratten B.
Ilford ortho-process plate.
Exposure, ½ second.
Arc lamp with Zeiss 1 c. collecting-lens.
Zeiss aplanatic condenser with top lens removed.

The main difficulty in such an object is to secure satisfactory detail and gradation in the structures shown. This is difficult to attain as the less dense surrounding tissue easily becomes over exposed. The selection of a screen of suitable density is the essential point. In some cases fluid screens are necessary as they can be varied in thickness and absorption.

PLATE XXI, FIG. 2.—**Thyroid Gland of Man.** × 67.

Stained bright red and blue.

Zeiss a.a. objective.

No ocular.

Camera-extension, 100 c.m.

Illumination similar to Fig. 1.

Although the same colour contrasts exist as in Fig. 1 the differences in density are not nearly so pronounced. Thus there is no difficulty in obtaining detail and gradation in all parts of the object.

PLATE XXI, FIG. 3.—**Cerebrum of Man.** × 67.

Optical conditions as Fig. 2.

The nerve fibrils are stained blue, the cells red and the ground a paler red.

Screen, pale green.

The elements of structure are shown on a nearly uniform ground. Correct exposure is essential to obtain almost black images of the deeply stained red cells, lightly stained blue fibrils and pale pink ground. Such objects are among the more difficult pathological preparations.

PLATE XXI, FIG. 4.—**Spinal Cord of Cat.** × 67.

Stained silver impregnation method of Golgi. Mounted in Canada balsam without cover-glass. Nerve fibrils black, ground yellow.

Yellow-green screen, chromic acid and copper nitrate solution.

Optical conditions as Fig. 2.

Such sections are usually thick and as they are mounted without cover-glass, the use of high powers is difficult. The surface of the balsam mount is irregular, which may interfere with the correction of the objective. Should an ocular be used, adjustment of tube-length can be attempted for the particular part of the object under observation.

PLATE XXI, FIG. 5.—**Auerbach's Plexus.** × 67.

This is not a section, but is a thin membrane mounted between slide and cover-glass. It is stained by a silver deposition process, the neuro-fibrils being a bluish-black.

Optical conditions as in Fig. 2.

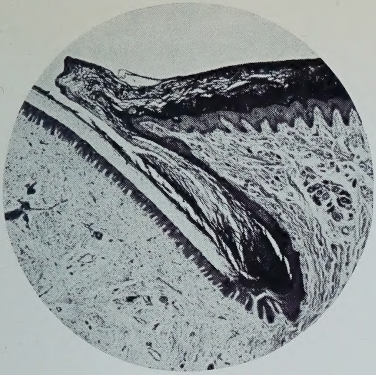


FIG. 1

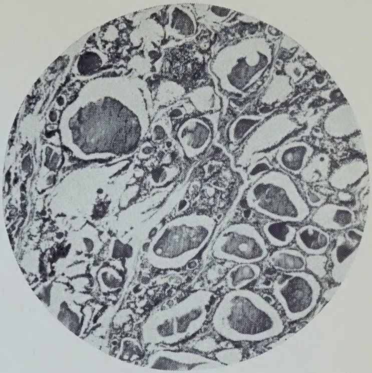


FIG. 2

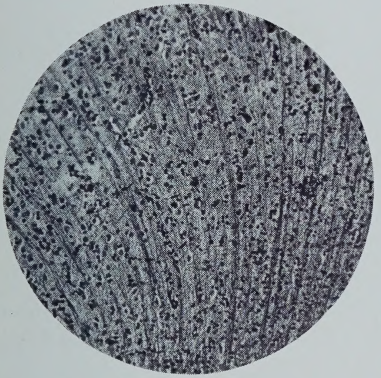


FIG. 3

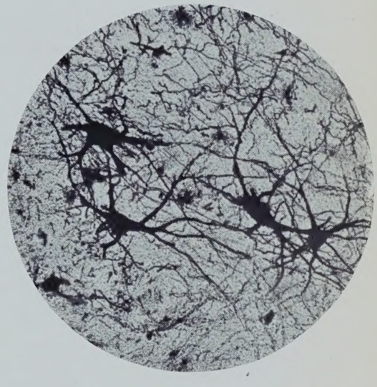


FIG. 4

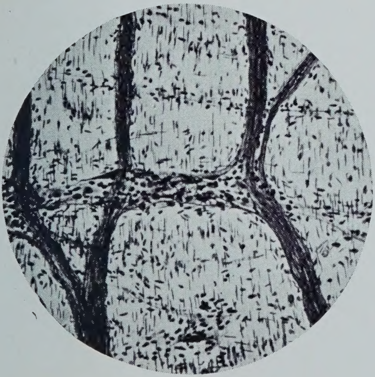


FIG. 5

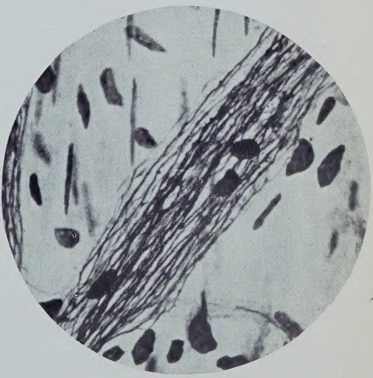


FIG. 6

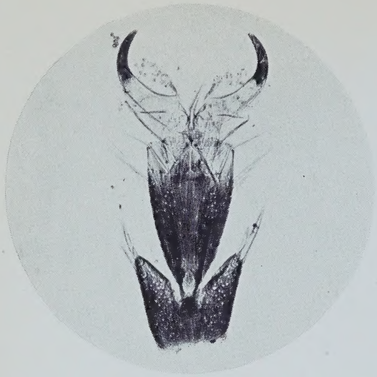


FIG. 1

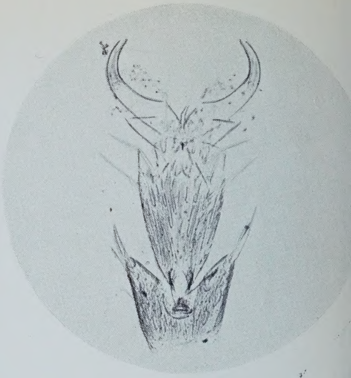


FIG. 2

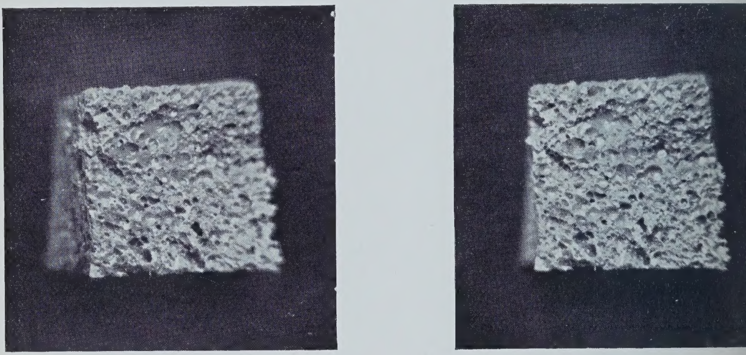


FIG. 3

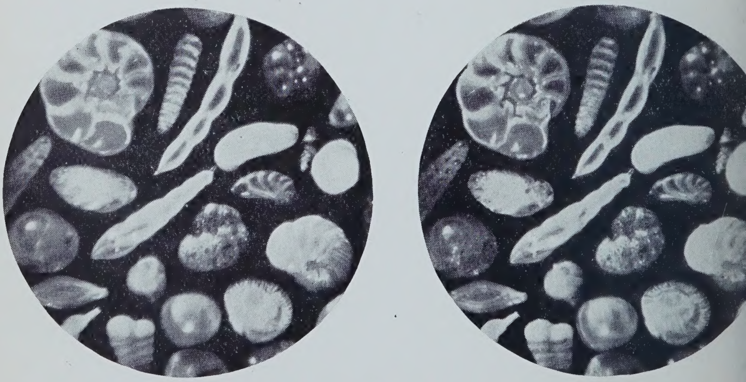


FIG. 4

Colour-screen, filter blue-green, aqueous solution.

It presents no particular difficulty, but the exposure must be full to ensure that the separate fibrils are shown.

Plate XXI, Fig. 6.—**Anerbach's Plexus.** A portion of Fig. 5. × 450.

Zeiss 8-mm. apochromat.

No. 4 projection ocular.

Camera-length, 100 cm.

The difficulty here is to obtain a satisfactory image of a bundle of fibrils not in one plane. To attain resolution, the largest practicable aperture of the objective must be utilised, but this should not be carried too far or most of the fibrils will be out of focus. Careful observation is necessary to decide the best combination of both sub-stage condenser and objective apertures.

Plate XXII, Fig. 1.—**Foot of Fly.** × 42. Photographed on Ilford soft gradation panchromatic plate.

Illuminant, Pointolite lamp.

Colour-screen, Wratten A (red).

Zeiss aspherical collecting-lens giving a parallel beam with secondary spectacle lens to converge the light on to the object.

No sub-stage condenser.

Zeiss a.a. objective, no ocular.

Exposure, $\frac{1}{2}$ second.

Plate XXII, Fig. 2.—**Foot of Fly.** Optical conditions and illuminant as Fig. 1. Photographed on Ilford infra-red plate to compare with Fig. 1.

Ilford infra-red colour-screen.

Exposure, $\frac{1}{2}$ second.

Both plates (Figs. 1 and 2) developed in pyro-soda and handled throughout in total darkness. It will be noted that the exposure is the same in each case, it was not necessary to increase exposure to infra-red.

Plate XXII, Fig. 3.—**Stereo-photo-micrograph.** Red sponge rubber. × 2.

Objectives, F = 55 mm.

Photograph taken by means of Greenough Binocular with Druner Camera.

The object was supported on a sheet of glass and black velvet was placed below out of the focus of the lens.

Illumination by 60-watt ‘Pearl’ electric lamp above and to one side of the object. White paper placed on opposite side as a reflector to reduce shadows.

Plate, Ilford Soft Gradation Panchromatic.

Colour-screen, Wratten F (red).

Exposure, 10 seconds.

Developer, pyro-soda.

PLATE XXII, FIG. 4.—**Stereo-photo-micrograph.** Foraminifera. × 24.

Objective, Zeiss a.a.

No ocular. Opposite halves of the objective were used as described on page 280. The object was focussed before the stop was inserted.

Illumination by 100-watt ‘Pearl’ electric lamp placed above and to one side of object. A small mirror was used to reduce shadows.

Plate, Ilford ortho-process.

Exposure, 5 minutes.

Zeiss vertical camera.

Ilford pyro-soda developer diluted to half-strength to reduce excessive contrast.

PLATE XXIII.—**Photo-micrographs with Ultra-violet Light.**

FIG. 1.—*Bacillus megatherium.* × 2000.

Dark-ground illumination with quartz cone condenser.

Objective, 1·7-mm. Zeiss monochromat.

Ocular, 7.

Wave-length, 275 $\mu\mu$.

Exposure, 10 seconds.

This photograph should be compared with Fig. 2, Plate XVI. The difference between the images obtained under the best conditions in visual light, when compared with this picture, will be obvious.

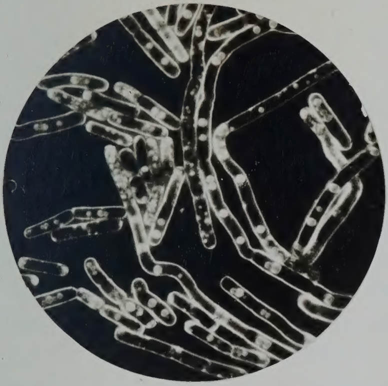


Fig. 1

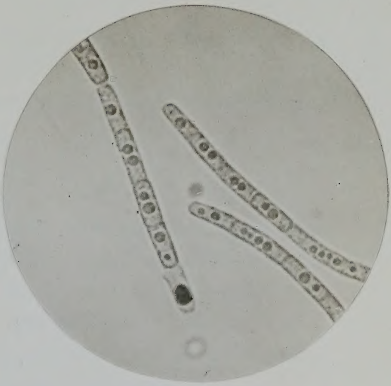


Fig. 2

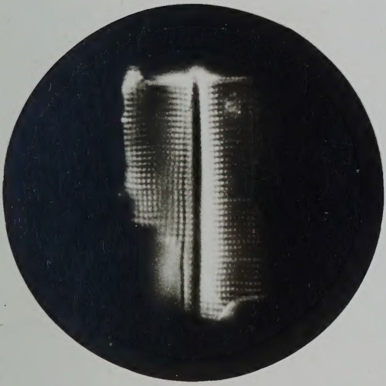


Fig. 3

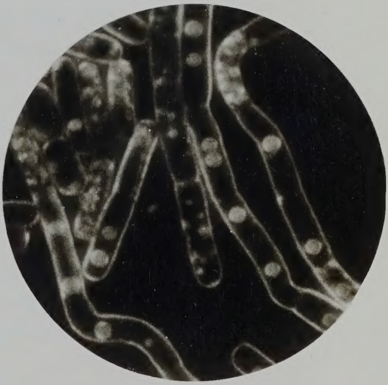


Fig. 4

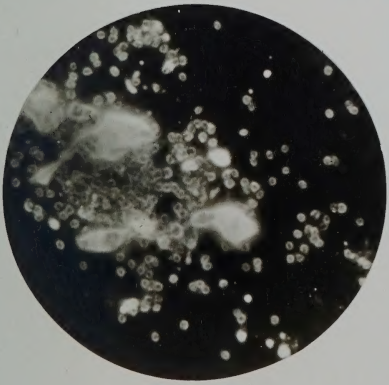


Fig. 5

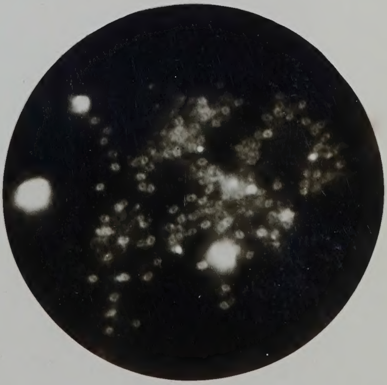


Fig. 6

FIG. 2.—Same preparation as Fig. 1 but photographed in transmitted light. × 2200.

Objective, 1·66-mm. Zeiss monochromat corrected for and used in wave-length 257 $\mu\mu$.

Ocular, 7.

Exposure, 1 second.

This is a particularly good result in transmitted light, and it is due to the greater visibility of the structure obtained by using a shorter wave-length. In spite of this, however, the dark-ground picture has a sharper appearance. Figs. 1 and 2 should be compared carefully to appreciate the advantages of each. They are in some respects complementary.

FIG. 3.—*Amphipleura pellucida.* × 2000.

Dark-ground illumination with quartz cone condenser.

Objective, 1·7-mm. Zeiss monochromat.

Ocular, 7.

Wave-length, 275 $\mu\mu$.

Exposure, 20 seconds.

This should be compared with Figs. 4, 5 and 6, Plate XVIII, to appreciate the increased resolution obtained.

FIG. 4.—Same as Fig. 1 but magnification × 3200.

This photograph should be used as a standard to compare with the size of the organisms shown in Figs. 5 and 6.

FIG. 5.—*Virus of Canary Pox.* × 3200.

A cytoplasmic inclusion body in process of disintegration so that the individual virus bodies are seen.

FIG. 6.—*Virus of Vaccinia.* × 3200.

In stained preparations it is difficult and generally impossible to distinguish between virus bodies and other particles of similar size. The ultra-violet photo-micrographs show them as definite bodies having the same characteristics as ordinary bacteria and differing from them only in size.

APPENDICES

I.—MICROSCOPIC AND SPECTROSCOPIC STANDARDS OF MEASUREMENTS

Micron ($1\ \mu$) = $\frac{1}{1000}$ millimetre (10^{-3} mm.) = 0·000039 inch.
Micro-millimetre ($1\ \mu\mu$) = $\frac{1}{1000}$ micron = 1 millionth of a millimetre.
Tenth-metre = 0·1 $\mu\mu$ = 10^{-10} metre.

II.—WEIGHTS AND MEASURES

The photographic formulæ have in most instances in this book been given in metric measures, but for convenience and as a matter of custom the English standards in certain cases have been used. The advantage of the metric system is obvious and needs no recommendation here.

Metric Unit of Weight is the gramme = 15·4 grains.
Decigramme = $\frac{1}{10}$ gramme = 0·1 grm. = 1·54 grains.
Centigramme = $\frac{1}{100}$,, = 0·01 ,, = 0·154 ,,
Milligramme = $\frac{1}{1000}$,, = 0·001 ,, = 0·015 ,,
Kilogramme = 1000 grammes = 1 kilo.
Millimetre (mm.) = $\frac{1}{1000}$ metre = 0·03937 inch.
Centimetre (cm.) = $\frac{1}{100}$,, = 0·3937 ,,
Metre (m.) = 39·37 inches.
Inch = 25·4 millimetres.
Cubic centimetre (c.c.) = $\frac{1}{1000}$ litre = 16 minims (approximate).
Litre = 1000 c.c. = 35 oz. ,,
Ounce = 28·35 grammes.

III.—REFRACTIVE INDICES

Approximate refractive indices of some substances occurring and used in microscopic work, for the D (sodium) line.

Air	1·0
Water	1·334
Alum (sat. sol.)	1·358
Absolute alcohol	1·368
Fluor-spar	1·438
Cedar-wood oil	1·510
Canada balsam	1·540
Styrax	1·582
Monobromide of naphthaline	1·657
Realgar	2·549
Crown glass	1·530 (about)
Flint glass	1·540 to 1·710

IV.—THE POSITION OF A PHOTOGRAPHIC LENS IN RELATION TO THE FOCUSSING-SCREEN OF THE CAMERA WHEN A DEFINITE DEGREE OF ENLARGEMENT OR REDUCTION OF AN OBJECT OR PRINT IS REQUIRED

The following short tables indicate the relative position of the lens and camera-screen in photographing certain microscopic objects. The measurements may be taken from the diaphragm of the lens, it being assumed that a symmetrical combination will be used and not an ordinary single achromatic lens. Should a single lens be used, the distance may be measured from the back surface of the lens.

	Number of Times of Enlargement.					
	1	2	3	4	5	6
Focus of Lens.	*Distance in Inches of Lens from Focussing-screen.*					
3 inches.	6	9	12	15	18	21
4 ,,	8	12	16	20	24	28
5 ,,	10	15	20	25	30	35
6 ,,	12	18	24	30	36	42
9 ,,	18	27	36	45	54	63

		Number of Times of Reduction				
		2	3	4	5	6
Focus of Lens.	*Distance in Inches of Lens from Focussing-screen.*					
3 inches.	6	$4\frac{1}{2}$	4	$3\frac{3}{4}$	$3\frac{1}{2}$	$3\frac{3}{7}$
4 ,,	8	6	$5\frac{1}{2}$	$4\frac{4}{5}$	$4\frac{2}{3}$	$4\frac{4}{7}$
5 ,,	10	$7\frac{1}{2}$	$6\frac{2}{3}$	$6\frac{1}{4}$	6	$5\frac{5}{6}$
6 ,,	12	9	8	$7\frac{1}{2}$	$7\frac{1}{5}$	7
9 ,,	18	$13\frac{1}{2}$	12	$11\frac{1}{4}$	$10\frac{4}{5}$	$10\frac{1}{2}$

V.—THE DETERMINATION OF THE N.A. OF MICROSCOPE OBJECTIVES

While an expert observer is often able approximately to estimate the N.A. of a microscope objective by direct observation of some known specimen, in practice some more exact method of determination is necessary. There are two appliances available, either of which is suitable for use with both dry and oil-immersion lenses; one is known as the Abbe Apertometer, and the other as Cheshire's Apertometer. There are other methods in use which are only applicable to dry lenses.

The Abbe appliance (Fig. 120) consists of a segment of glass about three inches in diameter and half an inch thick, the segmental portion of which is bevelled from above downwards and towards the semicircular edge, to an angle of 45°. Near the middle of the straight edge a disc of silvered glass is affixed, from the centre of which a small circle or strip of the silvering has been removed. Two sliding metal plates are so fitted on the semicircular edge that they can be moved round to any position. This edge of the plate is also graduated and marked with the full range of apertures up to 1·4, which represents practically the highest N.A. which is likely to be measured. To use the appliance the microscope is placed in a vertical position, and the glass plate is placed on the stage with the graduated surface uppermost. The objective, the N.A. of which is to be measured, is placed on the microscope and the hole or slit in the centre of the small glass disc is carefully focussed. An eye-piece which has a large field should be placed in position,

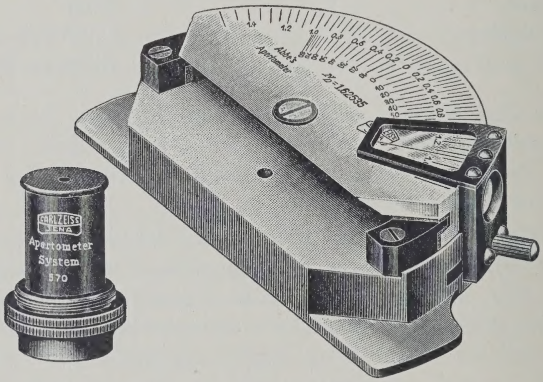


FIG. 120.—Apertometer, No. 155. (Full size.) (C. Zeiss.)

the general conditions under which the objective is to be used, such as the correct tube-length, etc., being duly observed.

With the apertometer an additional objective is supplied. The draw-tube of the microscope is now removed, and this objective is screwed into the bottom of the draw-tube, the eye-piece and the original lens being allowed to remain in position. The draw-tube is now returned to the body of the microscope, and is focussed down until the images of the two metal plates which slide round over the indices can be seen, these being moved round until they are in the field of view. It is important to see when returning the body-tube to the microscope that no alteration takes place in the position of the objective which is being measured. It must still remain in the position to which it was adjusted, to show with an ocular, and without the additional objective on the inside of the draw-tube, an image of the small spot in the centre of the silvered glass disc. The sliding metal pieces are carefully moved round until their edges just show on either side of the luminous disc as seen in the eye-piece. The reading of the indices may now be taken by direct observation, and if the objectives and eye-piece are exactly central and in alignment it will be found that the reading is the same on both sides, represents the exact N.A. of the objective. It may happen that some slight difference in the reading on either side results, and in this case the mean of the two readings may be taken, the result given being the exact N.A. The observing ocular must have a sufficiently large field to enable the whole of the field of view to be observed directly.

The makers give the following further instructions :

'To obtain a suitable illumination for the use of the apertometer the light should be made to fall in a horizontal direction upon the cylindrical sides of the glass plate. In the event of the source of light not being wide enough to send sufficient light to both pointers, so as to cause them to appear brightly illuminated at the same time, the difficulty may be overcome by placing in front of the microscope a semicircular translucent screen, e.g. an appropriately bent sheet of thin white paper. The upper edge of this screen should extend a little above the top of the apertometer.

'The power of the eye-piece used on the auxiliary microscope should rise with the magnifying power of the objective under examination, so as to obtain a sufficiently large image of the pointers, etc., but on no account should an eye-piece be used the diaphragm of which partly cuts down the bright circle of light as seen through the auxiliary microscope.

'To measure with the apertometer low apertures (less than 0·4 or 50°), which are generally associated with long foci of 7 mm.

and more, the pointer, etc., should be viewed with the unaided eye, without the auxiliary microscope. To this end in view the silver disc should be focussed, as described, and the eye-piece removed and replaced by a disc of cardboard or metal with a central hole. By this means the eye is kept in a central position whilst looking towards the objective.'[1]

The apparatus presents no unusual difficulty to a microscopist of even limited experience.

The Cheshire apertometer (Fig. 121) is somewhat more simple but it does not admit of such exact determinations with high apertures. It consists of a circular disc of glass with a mark on its upper surface, on which the objective to be measured is carefully focussed. The lower surface of the apertometer is ruled with a number of concentric rings, the distance between each corresponding to 0·1 N.A. In use the apertometer is placed on the stage of the microscope and the cross-lines ruled on the surface are brought into focus, the eye-piece is then removed and by direct observation the number of concentric circles in view can be counted: this gives directly the N.A. of the objective.

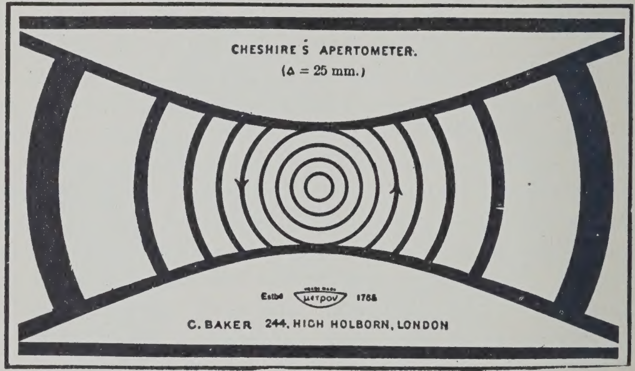


Fig. 121.

With high powers it is difficult to count the lines without some accessory observing appliance, since they appear so close together. A special eye-piece which focusses to the back focal plane of the objective may be obtained, and this is substituted for the ordinary eye-piece. The reading is then taken through the special eye-piece as before, and the aperture is indicated by the number of concentric circles that are visible.

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[1] See 'Directions for Use of Abbe Apertometer,' by Carl Zeiss.

VI.—FORMULA FOR PLATINISING GLASS

Make up the following solution:

Enamel collodion diluted with absolute alcohol and ether in equal parts	9 c.cs.
10 per cent. alcoholic solution platinum chloride	1 c.c.

The enamel collodion should be made very dilute so that it flows readily over a glass surface.

The glass to be coated should be chemically clean as for silvering. It must also be dry.

The glass is held by one corner and sufficient of the collodion-platinum chloride mixture to cover about one-third its area is poured on. The glass is tilted so that the solution runs over the whole surface and is drained back into the stock bottle, the glass being rocked from side to side until only a thin film of collodion remains and is then stood up on end to dry. The procedure is precisely the same as in coating a wet collodion plate and the same precautions as to cleanliness and even coating are necessary. When the film is thoroughly dry the glass is laid film side upwards on a metal plate and the whole heated over a bunsen flame. This must be done carefully to avoid fracturing the glass. The heating must continue until all the collodion is vaporised and a metallic coating of platinum remains. The glass should be allowed to remain on the metal plate until cold.

VII.—SEMI-SILVERING SOLUTIONS (Martin's process). From Baly: *Spectroscopy.*

Four solutions are made up.

A.	Silver nitrate	40 grammes.
	Distilled water	1000 c.cs.
B.	Ammonium nitrate	60 grammes.
	Distilled water	1000 c.cs.
C.	Potassium hydroxide (pure by alcohol)	100 grammes.
	Distilled water	1000 c.cs.

To make solution D, 100 grammes pure sugar candy are dissolved in 1000 c.cs. distilled water, and 23 grammes tartaric acid are added, after which the solution is boiled for 10 minutes. When cool 200 c.cs. alcohol are added and, lastly, distilled water to make the total volume up to 2000 c.cs.

Equal parts of A and B are mixed together, and similarly equal parts of C and D. Both these are mixed in the silvering vessel, and the mirror is at once suspended face downwards in the solution.

It is needless to point out the absolute necessity for perfectly clean surfaces; these may be obtained by rubbing the glass with a cotton-wool pad dipped in warm nitric acid and then thoroughly rinsing with water. A further treatment with alcohol, followed again by water, is also advisable. Opinions are widely at variance as to the best method of cleaning glass before silvering, and there is no doubt that different varieties of glass behave differently. In general a hard flint glass appears to be more suitable for such a purpose, perhaps because there is less surface action if acids are used for cleansing.

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